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ANALYTICAL PROCEDURES FOR ANILINE AND SELECTED DERIVATIVES IN WASTEWATER AND SLUDGE

by

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This study involved the development and validation of analytical methods for determining aniline and 18 halogen and nitro-substituted derivatives in industrial wastewater and municipal sludge. Gas chromatography (GC) with a nitrogen-phosphorus detector (NPD) was found to be well suited as a determinative technique for anilines in wastewater. Detection limits of 1-5 μg/L were achieved using both packed and capillary columns.

A GC-MS approach which was essentially the same as U.S. EPA Method 625 was found to be useful for identifying and determining anilines in industrial wastewater at the low μg/L level. A U.S. EPA Interim Method developed by EMSL-Cincinnati for the determinaton of priority pollutants in municipal sludge was evaluated and optimized for the determination of anilines. Detection limits of 50-300 μg/kg were achieved.

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory-Cincinnati, conducts research to:

- Develop and evaluate methods to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
- Investigate methods for the concentration, recovery, and identification of viruses, bacteria and other microbiological organisms in water; and, to determine the responses of aquatic organisms to water quality.
- Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.
- Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

Under authority of Sections 304(h) and 501(a) of the Federal Water Pollution Control Act of 1972 and the Clean Water Act of 1977, the Environmental Protection Agency is required to promulgate guidelines establishing test procedures for the analysis of pollutants. This report represents the state-of-the-art for the measurement of aniline and related derivatives in industrial and municipal wastewater and municipal sludge.

Robert L. Booth

Robert L. Booth, Acting Director
Environmental Monitoring and Support
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ABSTRACT

This study involved the development and validation of analytical methods for determining aniline and 18 halogen and nitro-substituted derivatives in industrial wastewater and municipal sludge. Gas chromatography (GC) with a nitrogen-phosphorus detector (NPD) was found to be well suited as a determinative technique for anilines in wastewater. Detection limits of 1-5 µg/L were achieved using both packed and capillary columns.

A GC-MS approach which was essentially the same as U.S. EPA Method 625 was found to be useful for identifying and determining anilines in industrial wastewater at the low µg/L level. A U.S. EPA Interim Method developed by EMSL-Cincinnati for the determination of priority pollutants in municipal sludge was evaluated and optimized for the determination of anilines. Detection limits of 50-300 µg/kg were achieved.

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SECTION 1

INTRODUCTION

The U.S. Environmental Protection Agency (U.S. EPA) under Section 304(h) of the Clean Water Act, as amended in 1977, has the responsibility of providing test procedures for the determination of organic pollutants in wastewaters. Two types of test procedures have been developed to date. One type is comprised of the specific 304(h) test procedures which are designed around relatively inexpensive equipment such as gas chromatography (GC) or high performance liquid chromatography (HPLC). These procedures are used for monitoring specific categories of pollutants. The other type is the screening protocol that is designed around more expensive equipment, combined gas chromatography-mass spectrometry (GC-MS), which is recognized as the most cost effective way of screening a wastewater or sludge for any wide variety of volatile and semivolatile organic compounds. Often data gathered by the GC-MS screening protocol are used to develop effluent limitations which need to be monitored by the specific 304(h) test procedure. Thus, there is an interrelationship of application as well as of chemistry for the two types of test procedures. The development of both should be unified under a single program, as was done in this study.

The specific objective of this program was to develop both monitoring and screening methods for determining a group of 19 aniline compounds in industrial wastewater as well as municipal sludge from publicly owned treatment works (POTW). These compounds, listed in Table 1, have been targeted for testing, and ultimate regulation, under the Toxic Substances Control Act (1).

The monitoring method for waste water was to be based on GC or HPLC with selective detectors whereas the screening method was to be based on GC-MS. If practical, U.S. EPA Method 625 (2) was to be employed as the screening method in order to reduce development costs. An existing U.S. EPA screening method for sludge (3) was to be evaluated, and modified if necessary, for determination of anilines in POTW sludge. This procedure, developed by the Environmental Monitoring and Support Laboratory (EMSL) of the U.S. EPA in Cincinnati, is presented in Appendix E.

This report describes the results of the development effort and presents the method performance data collected for relevant samples. A detailed description of the monitoring method, which employs GC with nitrogen phosphorus detection (GC-NPD), is given in Appendix A.

TABLE 1. ANILINE COMPOUNDS OF INTEREST

Aniline
2-Chloroaniline
3-Chloroaniline
4-Chloroaniline
4-Bromoaniline
3,4-Dichloroaniline
2,4,6-Trichloroaniline
2,4,5-Trichloroaniline
2-Nitroaniline
3-Nitroaniline
4-Nitroaniline
2,4-Dinitroaniline
4-Chloro-2-nitroaniline
2-Chloro-4-nitroaniline
2,6-Dichloro-4-nitroaniline
2,6-Dibromo-4-nitroaniline
2-Bromo-6-chloro-4-nitroaniline
2-Chloro-4,6-dinitroaniline
2-Bromo-4,6-dinitroaniline

SECTION 2

CONCLUSIONS

Based on the results presented in a subsequent section, several important conclusions may be drawn concerning the determination of anilines:

- U.S. EPA Method 625 can be used as a screening procedure for anilines with only minor modification. Wastewater samples should be adjusted to pH 6-8 when collected in order to stabilize the anilines. Furthermore, the packed column specified in Method 625 should be deactivated by repeatedly injecting a silylation reagent (e.g., BSTFA) until adequate peak shape is obtained for aniline at the level of interest. Use of a SE-54 fused silica capillary column (FSCC) has been found to be advantageous since the glass transfer line between the GC and MS can be eliminated and better resolution and sensitivity can be achieved. The performance of capillary columns for nitro substituted anilines, as described in Appendix A-Section 12, should be conducted prior to use of the columns for this application.

- The U.S. EPA Interim Method for GC-MS analysis of POTW sludge (Appendix E) can be successfully employed for the determination of anilines. The utilization of the Florisil cleanup and capillary column (SE-54) options were found to be appropriate. When utilizing capillary columns for sludge or wastewater analysis, the final extract should be dissolved in ethylene dichloride in order to obtain good column performance with splitless injection. However, when using the GC-NPD analysis procedure, toluene is a better injection solvent since chlorinated solvents readily degrade the NPD.

- The method presented in Appendix A, which was developed as a monitoring procedure for the determination of anilines in wastewater, was found to perform well for a number of relevant wastewaters. GC-NPD was found to be more sensitive, selective, and/or straightforward to operate than the photoionization or Hall (nitrogen selective mode) detectors.

- Although HPLC with ultraviolet (UV) and electrochemical (EC) detection were found to be useful for determining the anilines, these techniques did not prove to be as selective for anilines as GC-NPD when applied to authentic wastewater samples.

SECTION 3

RECOMMENDATIONS

The analytical methods presented in this report have been evaluated in a single laboratory using the relevant wastewater samples listed in Table 2. However, the methods should be evaluated in a number of different laboratories and for additional samples. This process will help to identify the range of applicability of the methods and will serve to define the interlaboratory method performance parameters.

When applying the GC-NPD method to sample types which previously have not been characterized, both the GC-MS and GC-NPD procedures should be employed initially in order to ensure that valid data can be obtained using the less specific monitoring procedure.

TABLE 2. DESCRIPTION OF RELEVANT WASTEWATERS USED IN THIS STUDY

Wastewater Designation	Chemicals Produced	Treatment Process Employed Prior to Discharge	Comments
1	Chloroanilines	None (not discharged into surface water)	
2	Chloronitroanilines	Sample taken before treatment	
2a	Chloronitroanilines	Activated sludge, clarification, settling pond.	Same facility as Wastewater 2.
3	Nitroanilines	Sample taken before treatment	
3a	Nitroanilines	Activated sludge, activated carbon, filtration.	Same facility as Wastewater 3.
4	Aniline	Activated sludge, clarification.	
Columbus (Ohio) POTW	--	--	Secondary sewage
Charles City (Iowa) POTW	--	--	Secondary sewage

SECTION 4

LITERATURE REVIEW

In the initial phase of this project a review was conducted of the available literature concerning analytical methods for the determination of anilines. The scope of this review was limited to chromatographic methods of analysis (i.e., electrochemical and colorimetric methods were not considered).

Chemical Abstracts and Water Pollution Abstracts were searched by a computer program using the key words "anilines" and "analysis" or "determination". Analytical Abstracts was searched manually. The following sections present the relevant information concerning GC and HPLC analysis.

GAS CHROMATOGRAPHY (GC)

A variety of GC approaches have been employed for anilines and related derivatives. In one study (4), 3% OV-17 on 100/120 mesh Supelcoport was used for eight of the anilines of interest in this study. These same workers found 3% OV-225 to be unsuitable due to peak tailings. Another study evaluated polypropylene glycol (LB-550-X) and silicone oil (MS550) for the separation of aniline and the three chloroanilines (5). All four compounds were separated on LB-550-X whereas 3- and 4-chloroaniline coeluted on silicone oil. This result indicated that Carbowax 20M-KOH might be a useful stationary phase in this program due to its similarity to polypropylene glycol. In fact, Carbowax 20M was found to be successful in separating five isomeric diaminotoluenes (6). Silar 10C has been found to give good peak shapes for aniline, diphenylamine, and several other aromatic amines (7). Excellent separation of the three nitroaniline isomers has been reported using cyclohexane-dimethanol succinate (8). QF-1 has also been used successfully for the separation of several chlorinated, brominated, and nitro-substituted anilines (9).

Both the electron capture detector (ECD) and NPD have been used to detect anilines. NPD has been shown to be useful for detecting diphenylamine with a sensitivity of ∿0.1 nanograms injected and has good selectivity for nitrogen containing compounds (10). Several studies have employed ECD, either with or without derivatization. In one study, the relationship between ECD sensitivity (for the underivatized compounds) and structure for several of the anilines was investigated (9). Nitro substituents were found to greatly increase EC response, whereas monochloro- or monobromoanilines were found to yield very low responses. The position of nitro or chloro substitution did not greatly affect the ECD response. Although the addition of multiple chloro groups did increase the ECD response, trichloroaniline was still 20 times less responsive than o-nitroaniline.

Consequently, it appeared that NPD may be the preferred detector for the anilines, since it yields good sensitivity, selectivity, and responds approximately the same to all of the anilines. However, ECD with derivatization is an alternative which could be explored if acceptable results are not obtained using direct GC separation. ECD has been used to detect aniline metabolites of carbamate and urea pesticides, using a variety of derivatization techniques (11). These authors concluded that pentafluoropropionyl anhydride was the derivatization reagent of choice since it gave a relatively clean GC background and good sensitivity (∿0.1 ng injected).

The Hall electrolytic conductivity detector (HECD) in the nitrogen selective mode has recently been used for the determination of anilines in sludge, and therefore should be considered as an alternative to NPD (12).

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Only a few papers have been published concerning the HPLC determination of anilines. Reversed-phase HPLC appears to be the method of choice for the anilines due to their polarity. At Battelle, a HPLC procedure has been developed for aniline and dimethylaniline using a Sperisorb ODS column and 60/40 methanol/aqueous phosphate buffer (pH 7) mobile phase. Two reports describe the use of reversed phase HPLC and EC detection to determine halogenated anilines in urine (13,14). A Zorbax ODS column and various combinations of acetonitrile and 0.15 M phosphate buffer, pH 2.1 was used. Good sensitivities (∿0.05 nanograms injected) were obtained for the various chlorinated anilines. However, at Battelle, the EC detector has been found to give relatively poor response for nitro-substituted anilines.

On the basis of the findings of this literature review, it appears that while HPLC with UV detection will give universal response for the 19 anilines, EC detection will give good response only for those anilines not containing nitro groups. Since UV detection is likely to give high background levels for complex wastewaters, it is advisable that HPLC be used primarily as a backup for the GC approach. However, both approaches were evaluated in this study in order to demonstrate the utility of each technique.

SECTION 5

EXPERIMENTAL PROCEDURES

WASTEWATER METHOD DEVELOPMENT

The wastewater method development tasks included evaluation of extraction, cleanup, and sample analysis by both HPLC and GC, as well as an evaluation of U.S. EPA Method 625 for determination of anilines in wastewater samples. Data obtained from these studies are presented in Section 6.

Sample Acquisition

Eight wastewater samples were collected for use in the aniline method development program. The samples, provided by chemical manufacturers and POTW treatment plant operators, were collected in five-gallon metal cans and stored at 4°C upon receipt by the laboratory. After an initial screening using U.S. EPA Method 625 (base/neutral extract), the samples were rebottled in either one-liter or one-gallon glass bottles with Teflon-lined screw caps and stored at 4°C until needed.

The wastewater samples obtained are described in Table 2.

Evaluation of GC

GC was evaluated as a technique for the determination of the aniline compounds. Both packed and wall-coated open tubular capillary columns were evaluated for the separation of the 19 compounds. Three specific GC detectors were evaluated. The gas chromatographs used throughout these evaluations were 1) a Hewlett Packard 5730A equipped with a flame ionization detector (FID) and NPD, 2) a Tracor Model 560 equipped with HECD, and 3) a Carbo Erba Model 2150 equipped with an HNU Model PI-50 photoionization detector (PID). The Hewlett Packard Model 5730A and FID was used for all column evaluation studies.

Packed Columns--

For the packed column work, the instrumental parameters were as follows:

• Column Dimensions:	6 ft x 2 mm ID glass
• Carrier Gas:	Helium at 30 mL/minute
• Injection Volume:	2 µL
• Solvent:	Methanol
• Solute Concentration:	50 µg/mL
• Attenuation:	1 x 16
• Temperature Program:	80° to 230°C at 4°C/minute.

The stationary phase and supports evaluated for the packed column work are listed below. These phases were selected to represent a wide range of polarities and treatment processes. Most of the column types deemed promising on the basis of the literature review described earlier were evaluated. However, since NPD was found most appropriate, certain nitrogen containing phases (e.g., Silar 10C) were not included in the evaluation. The nitrogen containing phases which were evaluated represented phases for which the evaluations were completed prior to selection of NPD as the detection system. All phases were coated on Supelcoport 100/120 mesh unless otherwise listed:

- 1.5% SP-2250/1.95% SP-2401
- 3% Silar 5 CP
- 3% SP-2250
- 10% SP-2250
- 5% SP-2401 DB
- 10% Carbowax 20M/2% KOH on Chromosorb WAW
- Ultrabond on Chromasorb WAW.

The data system used for retention time, peak area, and peak height determination was a Hewlett Packard 1000 minicomputer with the Computer Automated Laboratory System (CALS) software of Computer Inquiry Systems, Inc.

Capillary Column Studies--

For the capillary column work, the instrumental parameters were as follows:

- Column Dimensions: 30 m x 0.25 mm ID
- Carrier Gas: Helium at 2 mL/minute
- Injection Volume: 2 µL split
- Split Ratio: 10:1
- Solvent: Methanol
- Solute Concentration: 50 µg/mL
- Attenuation: 1 x 8
- Temperature Program: 80° to 230°C at 4°C/minute.

The glass capillary columns evaluated included SE-54 and SP-2250 (purchased from Supelco, Inc.) and SP-2100 and SE-52 (produced at Battelle). In addition, two FSCC columns (SE-54 and SE-30, both 30 m x 0.25 mm ID from J&W Scientific) were evaluated.

GC Detector Evaluation--

Three specific GC detectors were evaluated in this program: an NPD [sometimes referred to as an NP-FID, an alkali flame detector (AFD), or a thermionic specific detector (TSD)], an HECD operated in the nitrogen selective mode, and a PID using both the 9.5 and 10.2 eV ionization sources.

Detection limits were calculated from the peak height data using a 5:1 signal to noise ratio as the estimated minimum detectable signal.

Evaluation of HPLC

The HPLC evaluation was carried out in two phases, the first being the selection of optimum column and separation parameters, and the second the selection of the best detection technique.

HPLC Column Evaluations—

The instrumental conditions used in the column selection and separation optimization phase were as follows:

- Pump: Altex 110A
- Flow Rate: 1.0 mL/minute
- Injector: Micromeritics 725 Automatic sample injector equipped with a 20 μL sample loop
- Detector: LDC 1203 UV - III (254 nm)
- Sensitivity: 0.064 AUFS
- Recorder: Varian 9176 (10 mVFS)
- Data System: Spectra Physics 4000.

A total of seven HPLC columns were evaluated in the program. These included the following commercially available prepacked columns:

- Waters μBondapak C_{18} (10 μm)
- Dupont Zorbax ODS (5 μm)
- Perkin Elmer HC-ODS (10 μm)
- Whatman Partisil ODS/3 (10 μm)
- Lichrosorb RP-2 (5 μm)
- Spherisorb ODS (5 μm)
- Lichrosorb RP-18 (5 μm).

All columns were 250 mm x 4.6 mm ID except the Perkin Elmer HC-ODS, which was 2.6 mm ID. Two organic modifiers, methanol and acetonitrile, were evaluated for their effect upon the column selectivity. A variety of C-18 (or ODS) columns were evaluated, since differences in column selectivity between the various manufacturers can be significant.

The effect of mobile phase pH was evaluated using a sodium acetate-acetic acid buffer system (0.2 M) in the range of pH 4 to 5.5. The aqueous buffer of interest was combined with the organic modifier in the appropriate amounts to give a mobile phase of the desired composition. The mobile phase was filtered through a 0.2 μm polyester filter (Nucleopore) before use in the liquid chromatograph. Prior to injection of samples, the mobile phase was pumped through the column for at least 20 minutes to equilibrate the system.

HPLC Detector Evaluation--

Two HPLC detectors, the variable wavelength UV and the EC were evaluated in this program.

UV Detector--The instrument used in this evaluation was an LDC Spectromonitor III UV detector, which is equipped with a deuterium lamp. The wavelengths at which the aniline compounds were evaluated were 254 nm, 280 nm, and 340 nm. Detection limits were estimated at these wavelengths, and the linearity of response for the detector was evaluated for 254 nm and 340 nm. Prior to these determinations, the UV spectra of the aniline compounds in methanol solution were obtained over the range of 200 to 400 nm using a Cary 118 spectrophotometer.

EC Detector--The EC detector employed in this study was a Bioanalytical Systems LC-2A with a TL-5 glassy carbon electrode. The estimated detection limits of the aniline compounds were measured, as described above for GC, at +0.90 V, +1.10 V, and +1.30 V versus Ag/AgCl, and the linearity of response was measured at the +1.10 V potential.

Prior to the detection evaluation, the electrochemical oxidation potentials of the aniline compounds were determined by cyclic voltammetry using a Bioanalytical Systems DCV-3 potentiostat. A glassy carbon working electrode (CPAR Model 9333), platinum counter electrode, and Ag/AgCl reference electrode were used in these determinations. The voltage was scanned from 0 to 1.5 volts at a rate of 0.25 volts/second. Peak potentials were measured manually.

Extraction Techniques

The extraction efficiencies of the aniline compounds were determined in reagent water buffered at pH 5, 7, and 11 using separatory funnel extraction with a methylene chloride. Sample volumes of 1 liter and three serial 60-mL aliquots of methylene chloride were used for all extractions. The extraction was carried out using a two-liter separatory funnel and the combined methylene chloride extracts were collected in a 250-mL Kuderna-Danish concentrator. Following concentration to approximately five mL, a five-mL volume of acetonitrile was added and the extract was concentrated to a final volume of 0.1 mL using micro Kuderna-Danish techniques. The pH 5 buffer was prepared by adding 17.7 g sodium acetate trihydrate and 4.0 mL glacial acetic acid to 4 liters of deionized water. The pH 11 buffer was prepared by adding 1.31 g sodium hydroxide and 28.4 g dibasic sodium phosphate to 4 liters of deionized water. The pH 7 solution was prepared by dropwise addition of 10% phosphoric acid and 10% of sodium hydroxide to deionized water to achieve the proper pH (monitored with a pH meter). The compounds were spiked into the reagent water at the 10 µg/L level, with quantification of the extracts by HPLC with UV detection at 254 nm using the conditions shown in Figure 6. Other solvents were not evaluated since adequate recoveries were obtained and methylene chloride is commonly used in other U.S. EPA methods.

Sample Cleanup Techniques

Both acid back-extraction and adsorption chromatography on Florisil and alumina were evaluated as extract cleanup techniques.

Acid Back Extraction--

Mixtures of aniline standards in methylene chloride were equilibrated with equal volumes of 1 N aqueous sulfuric acid. After equilibration, the two phases were analyzed by HPLC with UV detection at 254 nm, using the conditions stated in Figure 6.

Adsorption Column Chromatography--

Alumina (Fisher Adsorption Grade, 80-200 mesh), basic alumina (Brockman Activity I, 80-200 mesh), and Florisil (PR Grade, 60-100 mesh) were evaluated using various elution schemes described below. Alumina and basic alumina were purchased from Fisher Scientific and Florisil was purchased from Sigma Chemical Company.

The anilines were introduced onto the adsorbent columns in the following manner. A one milliliter aliquot of a standard containing 50 μg/mL of each aniline compound in methanol and two grams of adsorbent was placed in a 50 mL beaker and the mixture was dried thoroughly under a gentle stream of nitrogen before addition to the top of the chromatographic columns. The beaker was rinsed with a 5 mL portion of the first elution solvent and the rinse added to the column.

Basic alumina--This adsorbent was evaluated using two solvent elution schemes as described below. The alumina was conditioned at 450°C overnight. Initial experiments showed the alumina to contain a large amount of impurities (using HPLC) with the elution scheme used, and it was necessary to extract the alumina in a Soxhlet extractor for 16 hours with CH_2Cl_2 and reactivate at 160°C for 16 hours before further use.

The first experiment performed with the extracted alumina was conducted with a hexane-diethyl ether-methanol elution scheme:

Fraction 1: 50 mL hexane (discarded)
Fraction 2: 50 mL 15% diethyl ether in hexane
Fraction 3: 50 mL 50% diethyl ether in hexane
Fraction 4: 100 mL 100% diethyl ether
Fraction 5: 100 mL 10% methanol in diethyl ether.

Twenty grams of basic alumina was placed in a 20 x 400 mm chromatography column which contained 70-80 mL of hexane, and the column tapped gently to settle the alumina as the excess hexane was eluted. Aniline standards (50 μg) were loaded onto the column in the manner dsecribed above. The column was eluted with the various solvents. Flow rates of 2-3 mL/minute were used in all experiments. The fractions were concentrated using Kuderna-Danish apparatus, exchanged to CH_3CN, and concentrated to 1.0 mL for HPLC analysis.

In order to further reduce the level of impurities and to lower the activity of the basic alumina, a second experiment was performed in which the adsorbent was packed in 10% methanol in diethyl ether and eluted with 50 mL of 100% ether followed by 50 mL of hexane. The 19 aniline compounds (50 μg each) were loaded onto the column and the same elution scheme was used to elute the column.

Adsorption alumina--Adsorption alumina was conditioned at 450°C in a muffle furnace overnight before use. The activated alumina (20 g) was placed in a 20 mm ID x 400 mm chromatography column containing 100 mL of diethyl ether. The column was tapped gently as the solvent eluted in order to settle the bed. The column was washed with 100 mL of hexane before loading the aniline compounds (25 μg each). The column was eluted with the following fractions:

Fraction 1: 50 mL hexane (discarded)
Fraction 2: 50 mL 15% diethyl ether in hexane
Fraction 3: 50 mL 50% diethyl ether in hexane
Fraction 4: 100 mL diethyl ether
Fraction 5: 100 mL diethyl ether containing 1% trimethylamine

The fractions were processed as described for previous studies and analyzed by HPLC. Trimethylamine was added to the last fraction in an attempt to increase the elution strength of the mobile phase for basic compounds.

Florisil--Florisil was extracted in a Soxhlet extractor overnight with methylene chloride and activated overnight at 160°C before use. Initially, three conditions were studied:

- 1% deactivated Florisil (as described in Appendix E)
- Activated Florisil using an elution scheme similar to that described in Appendix E
- Activated Florisil using a hexane/ether solvent series.

All columns were prepared by adding 20 grams of Florisil to a 20 mm x 400 mm column filled with hexane, and tapping the column to settle the Florisil while the hexane was eluting.

Water deactivated (1%) Florisil was prepared by adding 1% (by weight) deionized water to the activated Florisil, in an eight ounce jar, capping, and tumbling for at least four hours prior to use. The fractions used to elute the Florisil were:

Fraction 1: 50 mL hexane (discarded)
Fraction 2: 50 mL 10% CH_2Cl_2 in hexane
Fraction 3: 50 mL 50% CH_2CL_2 in hexane
Fraction 4: 150 mL 5% acetone in CH_2Cl_2.

Fractions were collected in 250 mL Kuderna-Danish apparatus, concentrated, exchanged to CH_3CN, and concentrated to a final volume of 1.0 mL. All samples were analyzed by HPLC.

Activated Florisil (160°C overnight) was evaluated as described above using the following elution scheme:

Fraction 1: 20 mL hexane (discarded)
Fraction 2: 20 mL 10% CH_2Cl_2 in hexane
Fraction 3: 25 mL 50% CH_2Cl_2 in hexane

Fraction 4: 25 mL 100% CH_2Cl_2
Fraction 5: 50 mL 5% acetone in CH_2Cl_2.

The 100% CH_2Cl_2 fraction was added to better define the elution characteristics of the various compounds.

Activated Florisil was evaluated using a second elution scheme, described below:

Fraction 1: 50 mL 6% diethyl ether in hexane
Fraction 2: 50 mL 15% diethyl ether in hexane
Fraction 3: 50 mL 50% diethyl ether in hexane
Fraction 4: 50 mL 100% diethyl ether.

An alternative deactivation procedure (called "isopropanol deactivation" procedure in Section 6) for Florisil was evaluated. This procedure involved packing 20 grams of activated Florisil into a column filled with 5% isopropanol in hexane (100 mL), and eluting the column with 50 mL of hexane. The anilines (5 µg each) were applied to the column and eluted with the following solvents:

Fraction 1: 75 mL hexane
Fraction 2: 50 mL 10% CH_2Cl_2 in hexane
Fraction 3: 50 mL 50% CH_2Cl_2 in hexane
Fraction 4: 200 mL 5% isopropanol in CH_2Cl_2
Fraction 5: 200 mL 5% methanol in CH_2Cl_2.

The various fractions were analyzed by HPLC.

Sample Preservation

Sample preservation studies were carried out in two phases. The specific samples included in each phase are described with the results in Section 6. In the first phase, six water samples were spiked at two levels and stored at pH 2 and 4°C for seven days. After a determination of the anilines by the method described in Appendix A, several samples were chosen for further study. These samples were evaluated for the effect of pH, extraction time, nitrogen purging, sodium thiosulfate addition, and sodium hypophosphite addition. The second phase of the storage stability experiments was carried out at pH 7 and 4°C for storage periods of 0 and 7 days. The effect of chlorination and addition of an organic solvent (toluene) as a preservative was also evaluated in this study.

GC Method Validation

The method validation consisted of the determination of the method detection limit (MDL) in four water samples, the determination of the analytical curve in reagent water, and high level spike evaluation in three water samples. The analytical procedure employed was identical to that described in Appendix A.

Method Detection Limit--

The MDL for the method was determined in reagent water, two relevant wastewaters, and a POTW effluent in accordance with the U.S. EPA/EMSL-Cincinnati protocol (Appendix B). The statistical basis for this approach is described in the literature (15). The determinations in reagent water were done using two groups of aniline compounds due to several coelutions in the GC analysis. For coeluting analytes in the wastewater samples, equal response and recovery were assumed.

Analytical Curve--

The analytical curve determinations consisted of replicate analyses of reagent water samples spiked over the range of approximately 3 to 300 times the MDL of the method, in accordance with the U.S. EPA/EMSL-Cincinnati protocol (Appendix C). As in the MDL determination, these analyses were carried out in two groups of compounds in order to circumvent coelution problems in the GC analysis of the samples.

High Level Spikes--

Reagent water and two relevant wastewater samples used in the MDL determinations were spiked at levels at least 10 times those used in the MDL experiment. Seven replicate determinations of each water sample were processed using the method described in Appendix A. As in the MDL experiment, the reagent water samples were analyzed in two groups due to GC coelutions. However, only a single group was used for the wastewaters in order to conserve sample.

U.S. EPA Method 625 Evaluation

U.S. EPA Method 625 was evaluated by analyzing in triplicate, three wastewater samples spiked at 10 times the estimated detection limit. The base/neutral methylene chloride extract was used in all determinations, and samples were analyzed using both a 3% SP-2250 packed column and the SE-54 FSCC. D_{10}-anthracene was used as the internal standard in all determinations. A Finnigan Model 3200 mass spectrometer was used for these studies. Prior to analysis, all extracts were exchanged into ethylene dichloride using the Kuderna-Danish apparatus. Chromatographic parameters for the packed column were as follows:

• Column:	6 ft x 2 mm ID glass; packed with 3% SP-2250 on Supelcoport (100/120 mesh)
• Carrier Gas:	Helium at 20 mL/minute
• Temperature Program:	80°C, 2 minutes; 80°C to 230°C at 4°C/minute
• Injection Volume:	2-5 μL.

The chromatographic conditions for the capillary column were:

• Carrier Gas:	Helium at 23 cm/sec at 250°C
• Temperature Program:	50°C, 3 minutes; rapid increase to 80°C, hold 3 minutes; 80°C to 270°C at 4°C/minute
• Injection Volume:	2 μL, splitless

POTW SLUDGE METHOD EVALUATION

The U.S. EPA/EMSL-Cincinnati protocol, given in Appendix E, for the analyses of organic priority pollutants in sludge (3) was evaluated for the aniline compounds, and the method was validated according to U.S. EPA procedures for MDL and analytical curve determination given in Appendices B and C, respectively.

Sample Acquisition

Sludge samples were collected from Columbus Municipal POTW and Cincinnati POTW for use in the evaluation and validation of the sludge method. The samples were collected in one-gallon glass jars and stored at 4°C until analyzed. In both cases, treated (secondary) sludge was collected. The Cincinnati POTW receives influent from a large number of industrial facilities whereas the Columbus POTW receives primarily municipal waste influent.

Extraction and Cleanup Extraction

Extraction--

Samples were homogenized and extracted as described in the U.S. EPA/EMSL-Cincinnati method (Appendix E). Spikes were introduced into the centrifuge tubes as methanol solutions to enhance dispersion of the spikes in the samples. The spiking volumes were always less than or equal to 5 mL per 80 mL of sludge. The conventional spiking procedure of tumbling overnight was not employed because of concerns about the stability of the anilines in the sample matrix.

Sample Cleanup Extraction--

Gel permeation chromatography (GPC)--The GPC characteristics of the aniline compounds were determined using conditions specified in Appendix E. Each component was injected as a 10 mg/L stock solution in methylene chloride using the following instrumental parameters:

- Instrument: Analytical Biochemistry Laboratory Model GPC 1001
- Column: 25 mm ID x 650 mm glass, packed with Bio-Bead SX-3 (∿70 grams)
- Flow Rate: 5.0 mL/minute
- Mobile Phase: Methylene chloride
- Injection Volume: 5.0 mL
- Detector: Altex Model 150 UV Detector operated at 254 nm and 0.05/AUFS.

Retention times were determined using a strip-chart recorder.

Adsorption chromatography on Florisil--1% H_2O-deactivated Florisil was prepared as described in Appendix E. Fractions 3 and 4 from the elution scheme were collected, combined, and concentrated to approximately 0.5 mL using Kuderna-Danish concentration techniques. The extract was spiked with

20 µg of D_{10}-anthracene internal standard using a 10-µL aliquot of a 2 mg/mL methylene chloride standard solution, homogenized on a vortex mixer, and transferred to a 1.75-mL GC vial. The Kuderna-Danish tube was rinsed with approximately 1 mL of ethylene chloride, which was mixed thoroughly with the extract in the vial and stored at 4°C until GC-MS analysis of the extract.

GC-MS Methodology

A Finnigan 3200 GC-MS system interfaced to a Finnigan INCOS data system was used for all analyses. All GC-MS data were archived on 9-track magnetic tape using standard INCOS software. The GC was equipped with a Grob-design split/splitless injector. The column was a 30-meter x 0.25 mm ID fused silica capillary coated with SE-54 (approximately 0.25 µm film thickness) supplied by J&W Scientific, Inc. The column was brought through the GC-MS interface transfer oven to a point about 1.5 mm from the entrance to the ion source, thus eliminating all transfer line connections. Since the column inlet was inserted into the injector evaporator cavity (i.e., no external connection was used), sample constituents encountered only the environment of the GC column from injector vaporization to MS source ionization.

The GC instrumental conditions employed were as follows:

- Injection Solvent: Ethylene dichloride
- Injection Volume: 2 µL
- Injection Conditions: 275°C; 4 mm ID quartz liner; splitless mode, 45 second
- Carrier Gas: Helium at 23 cm/second at 240°C.

The MS conditions were as follows:

- Source Tuning: As per U.S. EPA DFTPP requirement, but including the additional requirement that m/e 51, 69, and 77 be 40% to 70% of m/e 298
- Emission Current: Approximately 0.3 milliamperes
- Multiplier Gain: 2×10^4 to 4×10^4 (approximately 1700 volts)
- Pre-amplifier: 10^{-7} Amp/Volt
- Scan Range: 40 to 300 amu
- Scan Time: 1.5 second/cycle.

The INCOS data acquisition conditions were as follows:

- Parameters:
  - Scan time up; 1.45 second
  - Scan time down; 0.05 second
  - Hold time up; 0.0 second
  - Hold time down; 0.0 second
  - Minimum peak width; 5
  - A/D threshold; 1
  - Minimum area; 50
  - Baseline; 0
  - Samples per peak; 10

• Data Acquisition:	Start at injection; filament on at 6 minutes; multiplier on at 7.5 minutes; acquire 1800 scans (i.e., acquisition stopped at about 240°C).

A minimum of two calibration standards were analyzed each day.

An automated reverse search/quantification routine, based on standard INCOS software, was used for data processing. Manual inspection of the data was performed whenever the search/quantification results suggested some uncertainty in the determination. The response factors used were average values from standards analyzed on the same day as the samples.

Sludge Method Validation

The validation of the POTW sludge method (Appendix E) for anilines consisted of a determination of the MDL using Columbus and Cincinnati POTW sludge samples, according to the U.S. EPA/EMSL-Cincinnati protocol given in Appendix B, analysis of seven replicates of Columbus POTW sludge, spiked at high levels, and a determination of the analytical curve in Columbus POTW sludge according to the U.S. EPA/EMSL-Cincinnati protocol given in Appendix C.

Equilibrium Distribution in Sludge

For the equilibrium distribution studies, 100-mL aliquots of sludge were equilibrated for one hour and 24 hours with the aniline compounds. Samples were spiked from a stock solution of anilines in methanol, and 0.5 mL of methylene chloride was added to the sludge to retard microbiological activity. After equilibration, the aqueous phase was separated from the solid phase by centrifugation at 2400 rpm (1700 x g) for 30 minutes. The aqueous phase was analyzed for anilines using the U.S. EPA Method 625 whereas the solid phase was analyzed as described above for other sludge samples.

SECTION 6

RESULTS AND DISCUSSION

WASTEWATER METHOD DEVELOPMENT

GC Evaluation

Column Evaluation--

Retention time data for the various anilines on packed and glass capillary columns are given in Tables 3 and 4, respectively. These data were collected using the conditions given in Section 5. Since 2,4,6-trichloroaniline was added to the list at a later date, it was not included in these evaluations.

As indicated in Table 3, the 10% Carbowax-2% KOH column gave poor performance in that many of the anilines apparently did not elute from the column. Subsequently, a chemically bonded Carbowax column (Ultrabond® distributed by Supelco) was evaluated. All of the anilines were eluted from the Ultrabond® column. However, very poor response was obtained for aniline and 4-bromoaniline (indicating loss of these compounds on the column) and a number of compounds coeluted (e.g., 3-chloroaniline, 4-chloroaniline, and 2,4,6-trichloroaniline; 3,4-dichloroaniline, and 2-nitroaniline; and 4-chloro-2-nitroaniline and 2,6-dichloro-4-nitroaniline).

The 3% SP-2250 column partially resolved all of the anilines; however, some peak tailing was evident, especially for aniline itself. The 5% SP-2401 DB (containing KOH) and 1.5% SP-2250/1.95% SP-2401 gave no improvement in peak shape, in spite of the greater polarity, compared to SP-2250. In addition, several compounds were not resolved on these columns.

The Silar 5 CP column partially resolved all of the anilines except 3- and 4-chloroaniline, and gave good peak shape for all of the anilines. However, since NPD detection was selected as the detection mode in the final method, the Silar phase was not studied further since it contains cyano functions and gave a high background on the NPD. Instead, SP-2250 was chosen as the best available packed column for determination of anilines.

All the glass capillary columns evaluated gave excellent peak shape for the compounds of interest. However, each of the columns failed to resolve at least one compound pair, as shown in Table 4. None of the columns completely resolved 3-chloro and 4-chloroaniline although SE-54 appeared to give the best separation of these two compounds. SE-54 was selected for use in the program, although other phases (e.g., SE-52, SP-2100) were also considered to be acceptable. These columns should be considered for use when components coeluting on SE-54 need to be quantified.

TABLE 3. RELATIVE RETENTION TIME FOR ANILINES ON VARIOUS PACKED GC COLUMNS

Component	Relative Retention Time on Stated Column				
	3% SP2250	3% Silar 5CP	5% SP2401DB	1.5% SP2250-1.95% SP2401	10% Carbowax-2% KOH
(1) Aniline	1.0 (4.4)^{a}	1.0 (4.2)	1.0 (3.0)	1.0 (1.8)	1.0 (12.5)
(2) 2-Chloroaniline	1.7 (7.6)	1.6 (6.6)	1.8 (5.3)	2.0 (3.6)	1.4 (17.0)
(3) 3-Chloroaniline	2.4 (10.5)^{b}	2.6 (10.8)^{g}	2.8 (8.4)^{g}	3.2 (5.8)^{b}	1.8 (22.7)^{g}
(4) 4-Chloroaniline	2.4 (10.7)^{b}	2.6 (10.8)^{g}	2.8 (8.4)^{g}	3.4 (6.1)^{b}	1.8 (22.7)^{g}
(5) 4-Bromoaniline	3.2 (14.0)	3.4 (14.2)	3.7 (11.2)	4.8 (8.7)	2.1 (26.1)
(6) 3,4-Dichloroaniline	4.0 (18.0)^{c}	4.4 (18.5)^{b}	5.2 (15.5)	6.8 (12.2)	2.5 (31.5)^{h}
(7) 2-Nitroaniline	4.1 (18.2)^{c}	4.7 (19.8)	5.5 (16.5)^{b}	7.3 (13.2)	2.5 (31.5)^{h}
(8) 2,4,5-Trichloroaniline	4.6 (20.5)	4.5 (18.7)^{b}	5.7 (17.2)^{b}	7.9 (14.3)	2.6 (32.2)
(9) 3-Nitroaniline	4.7 (21.0)	5.8 (24.5)	6.7 (20.1)	8.8 (15.9)	ND ND^{i}
(10) 4-Chloro-2-nitroaniline	5.3 (23.7)	6.0 (25.3)	7.5 (22.5)	10.0 (18.0)	ND ND
(11) 4-Nitroaniline	5.7 (25.2)	7.4 (30.9)	8.5 (25.5)	11.1 (20.0)^{c}	ND ND
(12) 2,6,-Dichloro-4-nitroaniline	5.9 (26.5)^{d}	6.4 (26.8)	8.1 (24.4)	11.5 (20.7)^{c}	ND ND
(13) 2-Chloro-4-nitroaniline	5.9 (26.7)^{d}	7.2 (30.3)	8.8 (26.5)^{h}	11.8 (21.2)^{c}	ND ND
(14) 2-Bromo-6-chloro-4-nitroaniline	6.5 (29.3)	7.0 (29.5)	8.8 (26.5)^{h}	12.8 (23.1)	ND ND
(15) 2,6-Dibromo-4-nitroaniline	7.3 (32.1)^{e}	7.6 (32.1)	9.6 (28.7)	14.2 (25.6)	ND ND
(16) 2-Chloro-4,6-dinitroaniline	7.4 (32.5)^{e}	8.6 (35.9)	10.5 (31.5)	15.0 (27.0)	ND ND
(17) 2,4-Dinitroaniline	7.8 (34.5)^{f}	11.8 (49.6)	11.9 (35.8)	16.2 (29.1)^{g}	ND ND
(18) 2-Bromo-4,6-dinitroaniline	8.0 (35.0)^{f}	9.5 (39.9)	11.1 (33.3)	16.2 (29.1)^{g}	ND ND

(a) Actual retention time in minutes given in parenthesis.
(b) These compounds only partially resolved.
(c) " " " " "
(d) " " " " "
(e) " " " " "
(f) " " " " "
(g) These compounds essentially unresolved.
(h) " " " "
(i) Not detected - did not elute within 60 minutes.

TABLE 4. RELATIVE RETENTION TIMES FOR ANILINES
ON VARIOUS GLASS CAPILLARY GC COLUMNS

Component		Relative Retention Time on Stated Column			
		SP2250	SE-54	SE-52	SP2100
(1)	Aniline	1.0 (5.4)^{a}	1.0 (3.7)	1.0 (4.6)	1.0 (3.5)
(2)	2-Chloroaniline	1.7 (9.2)	1.8 (6.6)	1.8 (8.3)	1.8 (6.3)
(3)	3-Chloroaniline	2.2 (12.0)^{b}	2.3 (8.6)^{b}	2.3 (10.5)^{b}	2.3 (7.9)^{b}
(4)	4-Chloroaniline	2.2 (12.1)^{b}	2.4 (8.7)^{b}	2.3 (10.6)^{b}	2.3 (7.9)^{b}
(5)	4-Bromoaniline	2.9 (15.6)	3.1 (11.4)	3.0 (13.6)	3.0 (10.4)
(6)	2-Nitroaniline	3.7 (20.1)^{c}	4.1 (15.0)	3.7 (17.2)	3.8 (13.4)
(7)	3,4-Dichloroaniline	3.7 (20.1)^{c}	4.2 (15.7)	3.9 (17.9)	4.1 (14.3)
(8)	2,4,5-Trichloroaniline	4.2 (22.6)	5.1 (18.7)	4.7 (21.4)	5.1 (17.7)
(9)	3-Nitroaniline	4.3 (23.2)	4.8 (17.9)	4.3 (19.7)	4.4 (15.5)
(10)	4-Chloro-2-nitroaniline	4.8 (25.9)	5.7 (21.0)	5.1 (23.3)^{c}	5.5 (19.1)
(11)	4-Nitroaniline	5.2 (28.0)	6.0 (22.2)	5.1 (23.4)^{c}	5.4 (18.8)
(12)	2,6-Dichloro-4-nitroaniline	5.3 (28.6)	6.5 (23.9)^{c}	5.8 (26.8)	6.5 (22.7)
(13)	2-Chloro-4-nitroaniline	5.5 (29.5)^{d}	6.5 (24.0)^{c}	5.7 (26.1)	6.2 (21.6)
(14)	2,6-Dibromo-4-nitroaniline	5.5 (29.5)^{d}	7.9 (29.2)	7.0 (32.3)	8.0 (27.9)^{d}
(15)	2-Bromo-6-chloro-4-nitroaniline	5.9 (31.8)	7.2 (26.6)	6.4 (29.6)	7.2 (25.3)
(16)	2-Chloro-4,6-dinitroaniline	6.4 (34.7)	7.9 (29.1)	6.9 (31.9)	7.7 (27.1)
(17)	2-Bromo-4,6-dinitroaniline	7.0 (37.6)	8.5 (31.6)^{d}	7.5 (34.5)	8.5 (29.6)
(18)	2,4-Dinitroaniline	7.0 (37.9)	8.5 (31.6)^{d}	7.2 (33.2)	8.0 (27.9)^{d}

(a) Actual retention time in parenthesis.
(b) These compounds partially resolved.
(c) " " " "
(d) These compounds essentially unresolved.

During the course of this program, FSCC columns of suitable quality became available. These columns have the advantage of durability and ease of use, compared to conventional glass columns; therefore, the SE-54 FSCC was evaluated for the anilines. At this point in the study, 2,4,6-trichloroaniline had been included in the study to raise the total number of anilines of interest to 19. Retention data for the 19 anilines on SE-54 FSCC are given in Table 5. Since two coelutions occur on this column an additional FSCC, SE-30, was evaluated for use as an alternate column. Retention data for the SE-30 FSCC are also given in Table 5. The SE-30 FSCC gave slightly better resolution for 3-chloro- and 4-chloroaniline and completely resolved the two compound pairs which coeluted on SE-54. However, the SE-30 FSCC failed to resolve 2,6-dibromo-4-nitroaniline and 2,4-dinitroaniline. Interestingly, the fused silica and glass capillary SE-54 columns gave different elution patterns for the various anilines (compare Tables 4 and 5).

Representative chromatograms are given in Figures 1, 2, and 3 for the 3% SP-2250, SE-54, and SE-30, respectively. Using both SE-54 and SE-30 FSCC all 19 anilines can be resolved. SE-54 was used as the primary column for the method validation effort although SE-30 probably would have been just as appropriate.

Detector Evaluation--

The NPD, HECD (nitrogen selective mode), and PID detectors were evaluated in terms of sensitivity using SE-54 capillary columns. Estimated detection limits were determined as described in Section 5. The detection limit data for 18 anilines (not including 2,4,6-trichloroaniline) are presented in Table 6.

The HECD and NPD detectors gave similar detection limits in several cases; however, the NPD was consistently more sensitive for many of the compounds. In addition, the NPD gave a more stable baseline and less day to day variation in response. The PID gave very low response for the nitro- and dinitroanilines when the 9.5 eV source was used. The detection limits were improved considerably when the 10.2 eV source was used. However, the higher energy PID source will detect virtually all aromatic compounds and therefore was not expected to be as specific for anilines as the nitrogen selective detection systems. In addition, relatively poor baseline stability was obtained.

The selectivity of each of the three detectors was evaluated using an extract from a POTW aqueous effluent (Charles City). The PID detector (10.2 eV) gave a very complex chromatogram with a high baseline level. The chromatographic response using NPD and HECD is shown in Figures 4 and 5, respectively. The component identities given in these figures were confirmed using GC-MS.

Comparison of the chromatograms in Figures 4 and 5 shows that the HECD and NPD give comparable selectivites for determining anilines in this wastewater. Given this, NPD was selected for use in this study because it was more selective than PID and gave better baseline stability and day to day repeatability than HECD.

TABLE 5. RETENTION DATA FOR ANILINES ON FUSED SILICA CAPILLARY COLUMNS

Compound	Relative Retention Time on Stated Column	
	SE-54	SE-30
Aniline	1.0 (7.5)[a]	1.0 (6.3)
2-Chloroaniline	1.6 (12.1)	1.1 (7.1)
3-Chloroaniline	1.9 (14.6)[b]	1.4 (9.0)[b]
4-Chloroaniline	2.0 (14.7)[b]	1.5 (9.1)[b]
4-Bromoaniline	2.4 (18.0)	1.9 (12.1)
2-Nitroaniline	2.9 (21.9)[c]	2.1 (15.6)
2,4,6-Trichloroaniline	2.9 (21.9)[c]	2.5 (16.3)
3,4-Dichloroaniline	3.0 (22.7)	2.6 (16.6)
3-Nitroaniline	3.3 (24.5)	2.9 (18.0)
2,4,5-Trichloroaniline	3.5 (26.3)	3.2 (20.4)
4-Nitroaniline	3.8 (28.3)[d]	3.4 (21.7)
4-Chloro-2-nitroaniline	3.8 (28.3)[d]	2.9 (22.0)
2-Chloro-4-nitroaniline	4.2 (31.2)	3.3 (24.8)
2,6-Dichloro-4-nitroaniline	4.3 (31.9)	3.5 (26.0)
2-Bromo-6-chloro-4-nitroaniline	4.6 (34.8)	3.8 (28.8)
2-Chloro-4,6-dinitroaniline	4.9 (37.1)	4.0 (30.1)
2,6-Dibromo-4-nitroaniline	5.0 (37.6)	4.2 (31.6)[c]
2,4-Dinitroaniline	5.1 (38.4)	4.2 (31.6)[c]
2-Bromo-4,6-dinitroaniline	5.3 (39.8)	4.5 (33.4)

(a) Actual retention time in minutes given in parenthesis.
(b) These compounds only partially resolved.
(c) These compounds essentially unresolved.
(d) These compounds essentially unresolved.

COLUMN: 3% SP2250 ON SUPELCOPORT (100/120 MESH)
PROGRAM: 80°C 4 MINUTES, 4°C PER MINUTE TO 230°C
DETECTOR: NPD

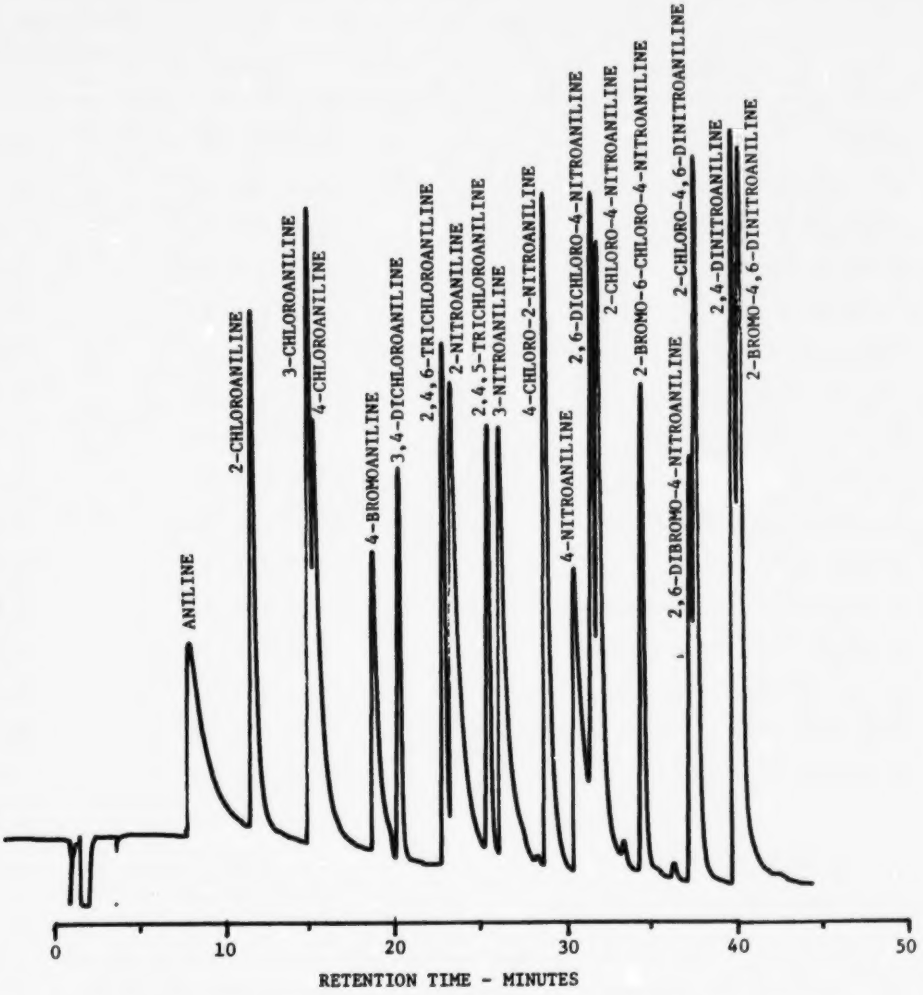

Figure 1. Gas chromatogram of aniline compounds on packed column.

COLUMN: SE-54 FUSED SILICA
PROGRAM: 80°C 4 MINUTES, 4°C PER MINUTE TO 230°C.
DETECTOR: NPD

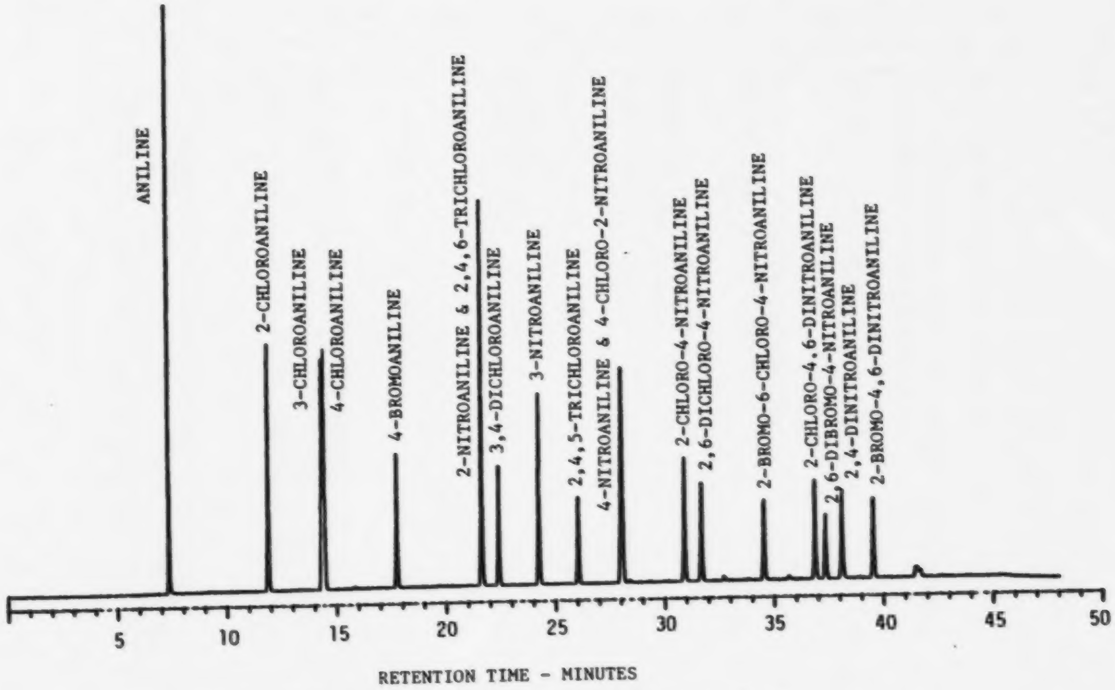

Figure 2. Gas chromatogram of aniline compounds on fused silica capillary column.

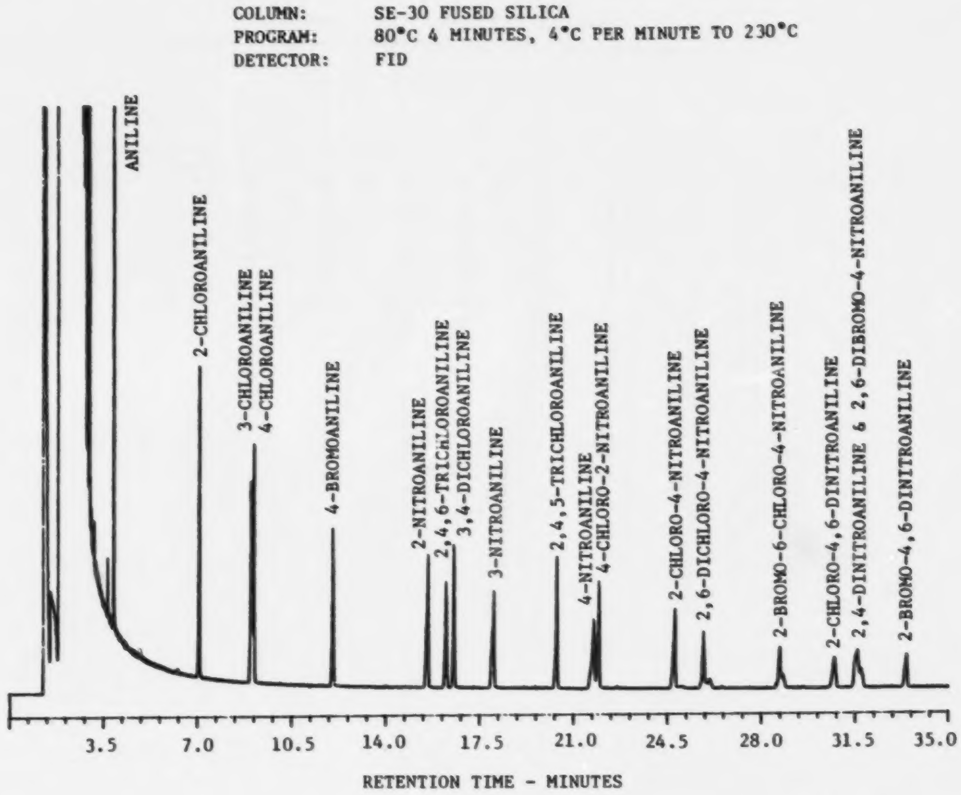

Figure 3. Gas chromatogram of aniline compounds on fused silica capillary column.

TABLE 6. ESTIMATED DETECTION LIMITS FOR ANILINES USING VARIOUS GC DETECTORS WITH GLASS CAPILLARY COLUMNS

	Estimated Detection Limit[a] (ng)			
Compound	Hall Detector[b]	NPD Detector[b]	Photoionization Detector[c] 10.2eV	9.5eV
Aniline	1.6	0.2	0.9	0.7
2-Chloroaniline	1.0	0.1	0.1	0.6
3-Chloroaniline	1.3	0.1	0.2	0.8
4-Chloroaniline	1.7	0.4	0.2	0.8
4-Bromoaniline	1.8	0.2	0.6	1.4
2-Nitroaniline	0.7	0.1	0.9	1.3
3,4-Dichloroaniline	1.8	0.4	0.6	1.1
2,4,5-Trichloroaniline	1.0	0.3	0.2	0.8
3-Nitroaniline	1.1	0.1	2	10
4-Chloro-2-nitroaniline	1.0	0.4	1	2.3
4-Nitroaniline	1.8	0.4	1	19
2,6-Dichloro-4-nitroaniline	0.7	0.4	2	30
2-Chloro-4-nitroaniline	1.5	0.3	2.5	11
2,6-Dibromo-4-nitroaniline	1.8	0.8	2.5	46
2-Bromo-6-chloro-4-nitroaniline	0.9	0.8	2.1	7.5
2-Chloro-4,6-dinitroaniline	2.2	1.5	10.4	100
2-Bromo-4,6-dinitroaniline	3.5	4.6	8.9	100
2,4-Dinitroaniline	12	5.0	21	100

(a) Estimated DL based on peak height that is 5 times the background noise level.

(b) DL determinations made on SE-54 glass capillary columns with 2 µl injections.

(c) DL determinations made on SE-52 glass capillary columns with 1 and 2 µl injections.

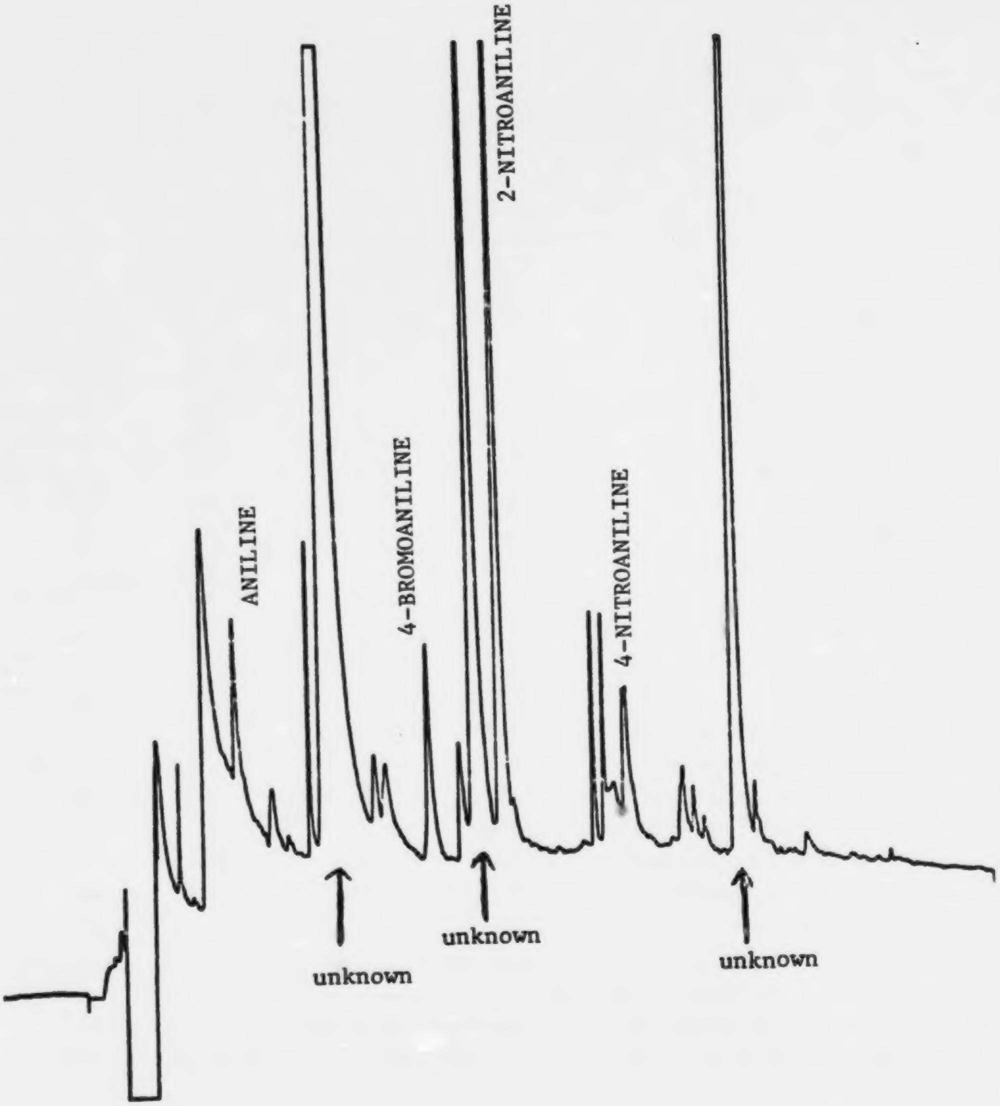

Figure 4. Gas chromatogram of unspiked POTW (Charles City) wastewater using an SE-54 capillary column and NPD.

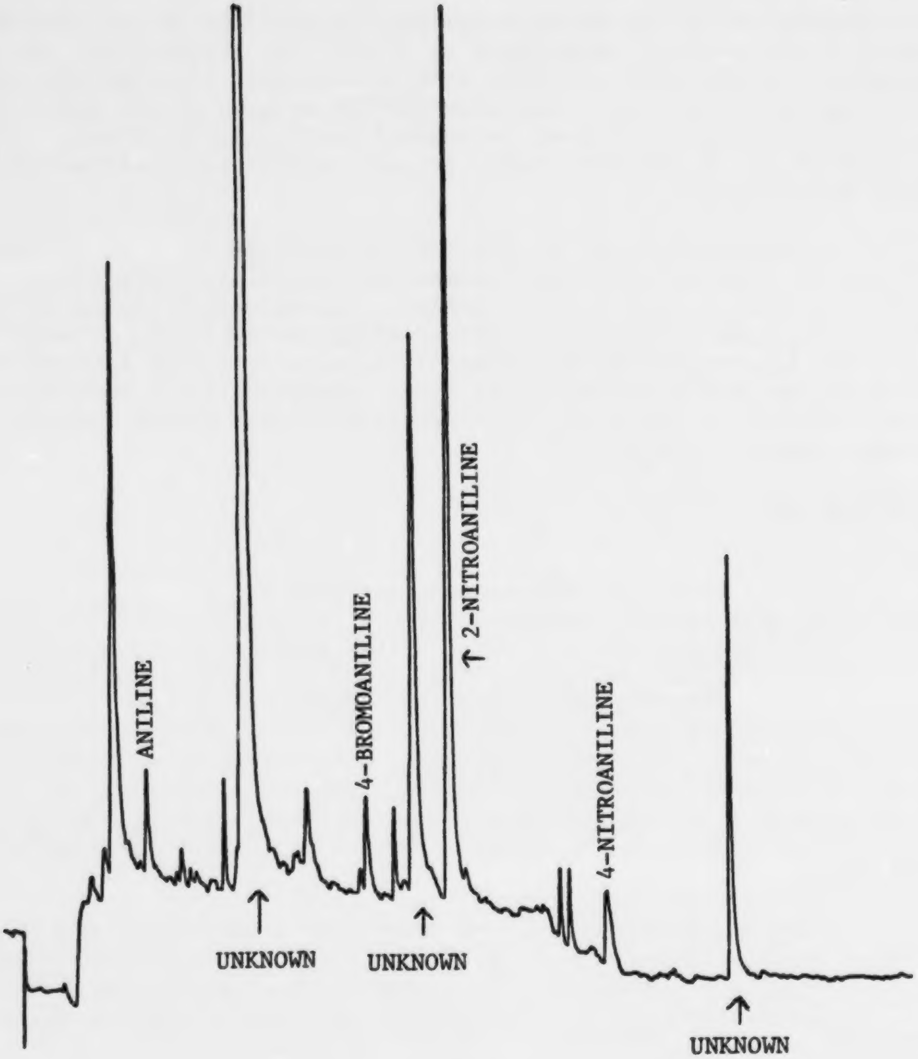

Figure 5. Gas chromatogram of unspiked POTW (Charles City) wastewater extract using an SE-54 capillary column and HECD.

The relationship between the NPD response and amount injected for the aniline compounds is presented in Tables 7 and 8. All of the anilines were detectable at the 10 ng level and all but the four latest eluting compounds could be detected at the 1 ng level. The response for the anilines was generally linear over the range 2-50 ng injected, although the response at the 2 ng level was higher than expected for several of the anilines (e.g., aniline, 3-chloroaniline, 4-bromoaniline, 2-nitroaniline). A linear least squares fit of the data (Table 8) gave correlation coefficients of 0.999 in most cases.

The sensitivity of the NPD for the anilines using 3% SP-2250 packed and SE-54 glass capillary columns was determined. These data are presented in Table 9. As shown in Table 9, the detection limits for many of the anilines are substantially better using the capillary column than they are for the packed column, primarily because less peak tailing is observed at low levels in the former case. Additionally, a more stable detector baseline is observed using capillary columns during temperature programmed runs.

HPLC Evaluation

Column Evaluation--

A large number of HPLC columns and mobile phase compositions were evaluated, as described in Section 5. Dupont Zorbax ODS was found to give the best resolution for the anilines. Better separation was obtained using 40% acetonitrile/60% acetate buffer than using 60% methanol/40% acetate buffer mobile phase. In addition, the column back pressure was substantially lower when acetonitrile was used. Adjusting the pH of the acetate buffer from 4 to 5.5, in 0.5 pH unit increments resulted in better separation between 2-chloroaniline and 2-chloro-4-nitroaniline (which coeluted at pH 4 and pH 4.5) but resulted in poor separation between 2-chloroaniline and 4-bromoaniline (which coeluted at pH 5 and pH 5.5). Buffer strength had little or no effect on the separation.

Based on these results, the optimum HPLC separation system was considered to be a Zorbax ODS column with a 40/60 acetonitrile/0.2 M sodium acetate buffer, pH 4. A typical chromatogram using this system is shown in Figure 6. This chromatographic system was employed for all subsequent HPLC evaluations.

Detector Evaluations--

Both UV and EC detectors were evaluated in terms of sensitivity for the anilines. UV absorption data for the anilines were collected using a Cary Model 118 spectrophotometer. Electrochemical oxidation potentials for the anilines were determined by cyclic voltammetry. The UV data and electrochemical data are presented in Tables 10 and 11, respectively.

As shown in Table 10, all of the nitro substituted anilines absorb strongly in the 340-400 nm region, whereas the other compounds show no appreciable absorbance above 300 nm. Consequently, the use of UV detection at 350 nm to selectively detect nitroanilines is feasible.

TABLE 7. RESPONSE FACTORS FOR GC QUANTIFICATION OF ANILINE COMPOUNDS USING NPD DETECTION

	Response Factor [a] Obtained From Injecting Given Amount, ng				
Compound	1	2	10	20	50
Aniline	638	1880	1070	1160	1200
2-Chloroaniline	968	1080	1240	1260	1360
3-Chloroaniline	797	2530	1210	1250	1250
4-Chloroaniline	425	630	930	1010	1200
4-Bromoaniline	381	1320	720	790	780
2,4,6-Trichloroaniline	b	b	b	b	b
2-Nitroaniline	1310	3140	1880	2010	2040
3,4-Dichloroaniline	365	443	683	737	847
2,4,5-Trichloroaniline	402	448	563	587	652
3-Nitroaniline	517	1410	1120	1290	1460
4-Chloro-2-nitroaniline	333	513	840	951	1110
4-Nitroaniline	160	640	570	720	760
2,6-Dichloro-4-nitroaniline	332	397	561	563	394
2-Chloro-4-nitroaniline	255	660	580	670	690
2,6-Dibromo-4-nitroaniline	c	220	200	240	220
2-Bromo-6-chloro-4-nitroaniline	165	220	366	366	243
2-Chloro-4,6-dinitroaniline	c	c	120	150	131
2-Bromo-4,6-dinitroaniline	c	c	46	58	71
2,4-Dinitroaniline	c	78	43	60	120

(a) Response factor in area counts/nanogram.
(b) Not determined.
(c) Not detected.

TABLE 8. LINEAR LEAST SQUARES PARAMETERS[a] FOR ANILINE COMPOUND CALIBRATION CURVES FROM GC ANALYSIS USING NPD DETECTION

Compound	Y-Intercept	Slope	Correlation Coefficient
Aniline	-196	1193	0.999
2-Chloroaniline	-989	1370	0.999
3-Chloroaniline	643	1230	0.999
4-Chloroaniline	-2010	1220	0.998
4-Bromoaniline	109	777	0.999
2,4,6-Trichloroaniline	(b)	(b)	(b)
2-Nitroaniline	-55	2040	0.999
3,4-Dichloroaniline	-1250	861	0.999
2,4,5-Trichloroaniline	682	659	0.999
3-Nitroaniline	-1880	1480	0.998
4-Chloro-2-nitroaniline	-1940	1130	0.999
4-Nitroaniline	-967	778	0.999
2,6-Dichloro-4-nitroaniline	982	395	0.982
2-Chloro-4-nitroaniline	-537	699	0.999
2,6-Dibromo-4-nitroaniline [c]	-4	221	0.999
2-Bromo-6-chloro-4-nitroaniline	690	244	0.975
2-Chloro-4,6-dinitroaniline [d]	108	130	0.996
2-Bromo-4,6-dinitroaniline [d]	-343	77	0.999
2,4-Dinitroaniline [c]	-654	126	0.980

(a) Calculated using nanograms injected as x-value and response as y-value with the five concentration levels shown in Table 6.

(b) Not determined.

(c) Three points only. Two lowest levels not detected.

(d) Four points only. Lowest level not detected.

TABLE 9. ESTIMATED DETECTION LIMITS FOR ANILINE COMPOUNDS ON PACKED AND CAPILLARY COLUMNS WITH NPD DETECTION

Compound	Detection Limit (nanograms injected)[a] 3% SP-2250, Packed	 SE-54 Glass Capillary
Aniline	4.4	0.2
2-Chloroaniline	2.2	0.1
3-Chloroaniline	2.2	0.1
4-Chloroaniline	2.5	0.4
4-Bromoaniline	4.2	0.2
3,4-Dichloroaniline	3.1	0.4
2,4,6-Trichloroaniline	2.5	b
2,4,5-Trichloroaniline	2.5	0.3
2-Nitroaniline	2.5	0.1
3-Nitroaniline	1.8	0.1
4-Nitroaniline	2.4	0.4
2,4-Dinitroaniline	5.0	5.0
4-Chloro-2-nitroaniline	1.3	0.4
2-Chloro-4-nitroaniline	1.8	0.3
2,6-Dichloro-4-nitroaniline	1.7	0.4
2,6-Dibromo-4-nitroaniline	2.2	0.8
2-Bromo-6-chloro-4-nitroaniline	2.0	0.8
2-Chloro-4,6-dinitroaniline	4.3	1.5
2-Bromo-4,6-dinitroaniline	5.3	4.6

(a) Detection limit based on peak height that is 5 times background noise level.

(b) Not determined

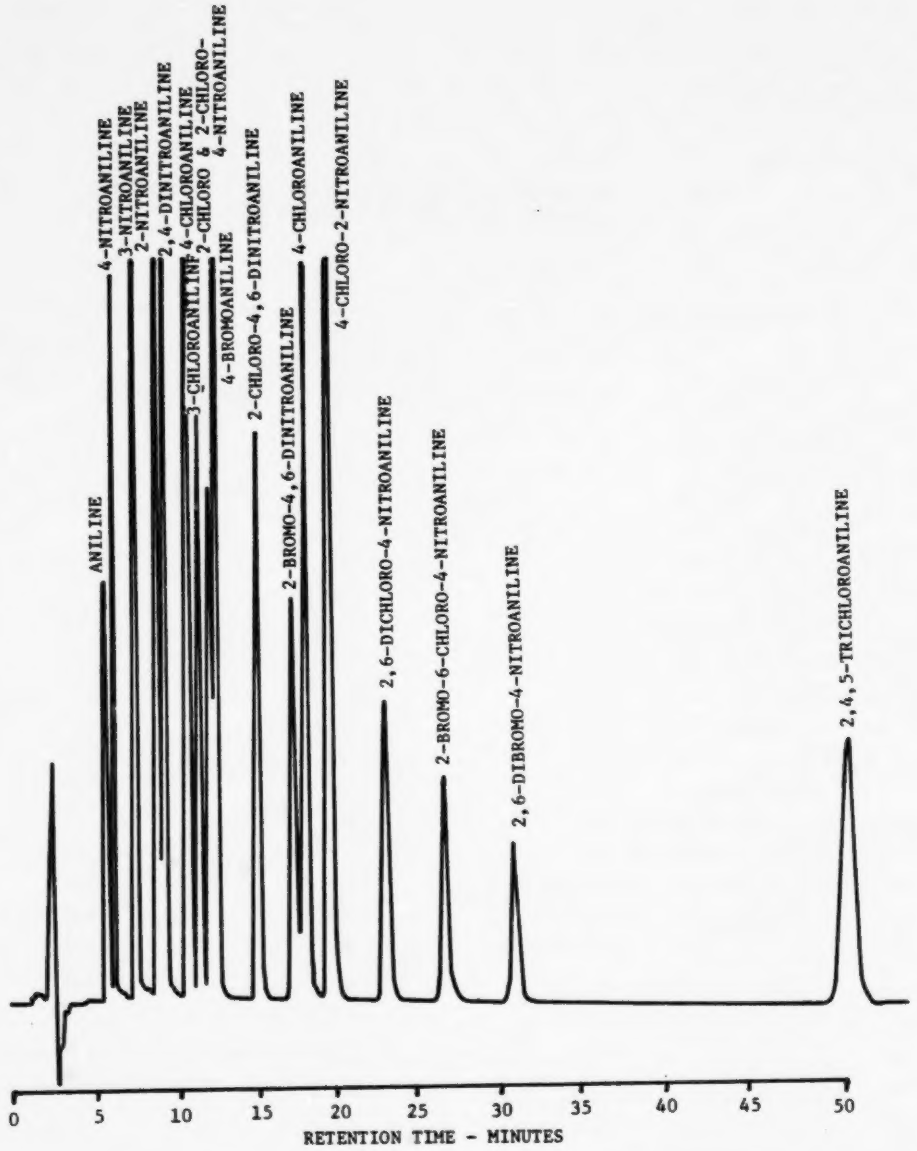

Figure 6. HPLC chromatogram of anilines on Zorbax ODS using a 40/60 acetonitrile/0.2 M sodium acetate (pH 4) buffer mobile phase at 1 mL/minute.

TABLE 10. UV ABSORPTION DATA FOR ANILINE COMPOUNDS

Compound	λ Max	ε x 10^3
Aniline	233	4.11
	284	0.77
4-Nitroaniline	373	16.8
	228	6.6
3-Nitroaniline	237	15.5
	374	3.4
	278 (sh)[b]	2.82
2-Nitroaniline	232	12.4
	402	8.9
	278	4.01
2,4-Dinitroaniline	339	15.0
	259	9.16
	228	10.3
4-Chloroaniline	244	1.65
	297	1.59
3-Chloroaniline	241	8.46
	292	1.78
2-Chloroaniline	237	7.97
	293	2.21
2-Chloro-4-nitroaniline	365	14.9
	225 (sh)	5.29
4-Bromoaniline	244	14.9
	298	2.18
2-Chloro-4,6-dinitroaniline	229	20.0
	264 (sh)	14.7
	334	13.2
2-Bromo-4,6-dinitroaniline	229	19.8
	268 (sh)	17.8
	334	12.4

TABLE 10. (continued)

Compound	λ Max	ε x 10^3
4-Chloro-2-nitroaniline	415	5.09
	230[a]	20[a]
3,4-Dichloroaniline	248	12.0
	307	2.06
2,6-Dichloro-4-nitroaniline	353	13.5
	246 (sh)	13.4
2-Bromo-6-chloro-4-nitroaniline	354	12.3
	253 (sh)	14.3
2,6-Dibromo-4-nitroaniline	351	12.8
	248 (sh)	17.1
2,4,5-Trichloroaniline	248	11.7
	313	2.85

(a) Estimated, peak was offscale.

(b) sh = shoulder

TABLE 11. CYCLIC VOLTAMMETRY DATA FOR ANILINE COMPOUNDS

Compound	Peak Potential (Volts vs. Ag/AgCl)
Aniline	0.95
4-Nitroaniline	1.17
3-Nitroaniline	1.07
2-Nitroaniline	1.20
2,4-Dinitroaniline	1.40
4-Chloroaniline	0.90
3-Chloroaniline	1.02
2-Chloroaniline	1.00
2-Chloro-4-nitroaniline	1.15
4-Bromoaniline	0.90
2-Chloro-4,6-dinitroaniline	1.42
2-Bromo-4,6-dinitroaniline	1.32
4-Chloro-2-nitroaniline	1.17
3,4-Dichloroaniline	0.97
2,6-Dichloro-4-nitroaniline	1.20
2-Bromo-6-chloro-4-nitroaniline	1.15
2,6-Dibromo-4-nitroaniline	1.17
2,4,5-Trichloroaniline	1.00

The electrochemical oxidation potentials for the anilines, shown in Table 11, ranged between 0.9 and 1.4 volts, with the dinitroanilines being the most difficult to oxidize. Estimated detection limits for UV and EC detection of the anilines are given in Table 12 and 13. As shown in Table 12, only the nitro-substituted anilines were detected at 340 nm, whereas all of the anilines were detected at 254 and 280 nm. In general, detection limits were 1-10 nanograms injected using UV detection, which corresponded to a level of 0.1-1 μg/mL in the sample extract.

As shown in Table 13, several of the dinitroanilines were not detected at 1.3 volts using the electrochemical detector. Since the selectivity of the electrochemical detector is diminished as the electrode potential is increased, 1.3 volts is considered to be the practical upper limit. Therefore, the electrochemical detector was not considered further in this program. Although a tandem system using an EC detector at 1.3 volts in series with a UV detector at 340 nm (to selectively detect the dinitro derivatives not detected by the EC detector) might be effective, such a system was considered too complex for the purposes of this program.

The linearity of response for the anilines using the UV detector at 254 nm was evaluated over the 5-500 nanogram (injected) range. These data and the corresponding linear least squares regression analysis data are presented in Tables 14 and 15, respectively. In all cases, the linearity was excellent, and correlation coefficients of 0.999 or greater were obtained.

Extracts from POTW (Charles City) wastewater were injected onto the HPLC-UV system with the UV detector successively at 254, 280, and 340 nm. In all cases, a very complex chromatogram was obtained. Although sample cleanup techniques could have been used in an attempt to reduce the background interference level, the evaluation of HPLC analysis was discontinued at this point because much better selectivity had been obtained using the GC-NPD system described previously.

Evaluation of Extraction Techniques

The extraction efficiency of the anilines at pH 5, pH 7, and pH 11 was initially evaluated using shake-out with methylene chloride described in Section 5. The recovery data from this experiment are presented in Table 16. Recovery ranged from 79-100% at pH 5, 86-111% at pH 7, and 79-100% at pH 11. The slightly greater recoveries observed at pH 7 compared to the other pH levels are considered to be within experimental error. Extraction at pH 11 was selected for the final method since this practice helps to eliminate phenolic and other acidic material that might interfere with the subsequent GC-NPD analysis. Additionally, this pH level is consistent with the base/neutral extraction employed in U.S. EPA Method 625. At this pH, all recoveries were greater than 85% except for aniline which gave a 79% recovery.

Evaluation of Cleanup Techniques

Two extract cleanup approaches, acid back extraction and adsorption chromatography, were evaluated. The data for the acid back extraction experi-

TABLE 12. DETECTION LIMITS OF ANILINE COMPOUNDS USING UV DETECTION AT VARIOUS WAVELENGTHS

Compound	Detection Limit in Nanograms Injected at Selected Wavelengths[a] 254 nm	280 nm	340 nm
aniline	5.3	3.1	ND[b]
4-Nitroaniline	1.4	10	0.65
3-Nitroaniline	0.7	1.4	5.3
2-Nitroaniline	1.5	1.3	7.1
2,4-Dinitroaniline	0.9	2.9	0.67
4-Chloroaniline	1.5	7.1	ND[b]
3-Chloroaniline	3.9	5.6	ND[b]
2-Chloroaniline	12.5	4.2	ND[b]
2-Chloro-4-nitroaniline	3.6	8.3	1.3
4-Bromoaniline	1.5	7.4	ND[b]
2-Chloro-4,6-dinitroaniline	2.2	3.3	1.4
2-Bromo-4,6-dinitroaniline	3.6	4.2	2.2
4-Chloro-2-nitroaniline	2.0	4.6	20
3,4-Dichloroaniline	2.0	15	ND[b]
2,6-Dichloro-4-nitroaniline	4.8	12	2.2
2-Bromo-6-chloro-4-nitroaniline	6.7	15	3.0
2,6-Dibromo-4-nitroaniline	8.3	18	4.2
2,4,5-Trichloroaniline	8.3	50	ND[b]

(a) Estimated DL based on peak height that is 5 times background noise level using a 10 μL injection of standard.

(b) Not detected at 50 nanograms injected.

TABLE 13. DETECTION LIMITS OF ANILINE COMPOUNDS USING ELECTROCHEMICAL DETECTION AT VARIOUS OXIDATION POTENTIALS

	Detection Limit in Nanograms Injected at Selected Potential[a]		
Compound	+0.90V	+1.10V	+1.30V
Aniline	0.040	0.13	0.30
4-Nitroaniline	ND[b]	5.0	0.80
3-Nitroaniline	ND	0.63	0.77
2-Nitroaniline	ND	ND	1.11
2,4-Dinitroaniline	ND	ND	ND
4-Chloroaniline	0.14	0.27	0.71
3-Chloroaniline	2.2	0.26	0.65
2-Chloroaniline	ND	ND	0.84
2-Chloro-4-nitroaniline	ND	ND	2.3
4-Bromoaniline	0.25	0.35	0.87
2-Chloro-4,6-dinitroaniline	ND	ND	ND
2-Bromo-4,6-dinitroaniline	ND	ND	ND
4-Chloro-2-nitroaniline	ND	ND	2.4
3,4-Dichloroaniline	5.9	0.53	1.5
2,6,-Dichloro-4-nitroaniline	ND	ND	6.7
2-Bromo-6-chloro-4-nitroaniline	ND	ND	9.1
2,6-Dibromo-4-nitroaniline	ND	ND	12.5
2,4,5-Trichloroaniline	ND	3.3	6.7

(a) Estimated DL based on peak height that is 5 times background noise level using a 10 uL injection of standard.

(b) Not detected at 50 nanograms injected.

TABLE 14. RESPONSE FACTORS FOR HPLC QUANTIFICATION OF ANILINE COMPOUNDS WITH UV DETECTION AT 254 nm

	Response Factor[a] Obtained From Injecting Given Amount, ng				
	5	20	100	250	500
Aniline	848	800	810	829	800
2-Chloroaniline[b]	760	650	650	650	650
3-Chloroaniline	1750	1800	1770	1800	1750
4-Chloroaniline	3610	3550	3550	3580	3500
4-Bromoaniline[b]	3500	3300	3330	3330	3280
2,4,6-Trichloroaniline	2600	2490	2390	2450	2380
2-Nitroaniline	2220	2260	2240	2280	2190
3,4-Dichloroaniline	4100	3890	3930	4010	3920
2,4,5-Trichloroaniline	3190	3140	3140	3200	3120
3-Nitroaniline	4990	4900	4870	4980	4860
4-Chloro-2-nitroaniline	3360	3300	3330	3390	3310
4-Nitroaniline	1490	1550	1480	1520	1480
2,6-Dichloro-4-nitroaniline	1680	1670	1640	1680	1620
2-Chloro-4-nitroaniline[b]	1490	1530	1490	1550	1490
2,6-Dibromo-4-nitroaniline	1220	1240	1210	1230	1200
2-Bromo-6-chloro-4-nitroaniline	1420	1380	1430	1450	1410
2-Chloro-4,6-dinitroaniline	2250	2180	2180	2220	2160
2-Bromo-4,6-dinitroaniline	1580	1480	1530	1560	1530
2,4-Dinitroaniline	3910	3850	3830	3910	3820

(a) Response factor in area counts/nanogram.
(b) Determined individually because of coelution.

TABLE 15. LINEAR LEAST SQUARES PARAMETERS [a] FOR ANILINE COMPOUND CALIBRATION CURVES FROM HPLC ANAYLSIS USING UV DETECTION AT 254nm

Compound	Y-Intercept	Slope	Correlation Coefficient
Aniline	1810	796	0.999
2-Chloroaniline	77	652	0.999
3-Chloroaniline	4474	1720	0.999
4-Chloroaniline	7330	3470	0.999
4-Bromoaniline	3230	3280	0.999
2,4,6-Trichloroaniline	5640	2350	0.999
2-Nitroaniline	4950	2190	0.999
3,4-Dichloroaniline	7920	3860	0.999
2,4,5-Trichloroaniline	7280	3070	0.999
3-Nitroaniline	9070	4810	0.999
4-Chloro-2-nitroaniline	6300	3270	0.999
4-Nitroaniline	3230	1460	0.999
2,6-Dichloro-4-nitroaniline	2370	1630	0.999
2-Chloro-4-nitroaniline	2020	1500	0.999
2,6-Dibromo-4-nitroaniline	2820	1180	0.999
2-Bromo-6-chloro-4-nitroaniline	1650	1410	0.999
2-Chloro-4,6-dinitroaniline	4780	2130	0.999
2-Bromo-4,6-dinitroaniline	2220	1510	0.999
2,4-Dinitroaniline	6620	3780	0.999

(a) Calculated using nanograms injected as x-value and response as y-value with the five concentration levels in Table 13.

TABLE 16. EFFICIENCY OF EXTRACTION OF 18 ANILINES FROM REAGENT WATER AT 10 µg/L LEVEL USING METHYLENE CHLORIDE

Compound	% Recovery[a]		
	pH 5	pH 7	pH 11
Aniline	86	86	79
4-Nitroaniline	98	95	94
3-Nitroaniline	91	96	91
2-Nitroaniline	90	107	87
2,4-Dinitroaniline	100	105	100
4-Chloroaniline	79	96	86
3-Chloroaniline	85	106	86
2-Chloroaniline	88[b]	101[b]	90[b]
2-Chloro-4-nitroaniline	88[b]	101[b]	90[b]
4-Bromoaniline	88[b]	101[b]	90[b]
2-Chloro-4,6-dinitroaniline	99	105	96
2-Bromo-4,6-dinitroaniline	91	110	93
4-Chloro-2-nitroaniline	94	111	94
3,4-Dichloroaniline	86	101	88
2,6-dichloro-4-nitroaniline	95	109	91
2-Bromo-6-chloro-4-nitroaniline	94	106	92
2,6-Dibromo-4-nitroaniline	98	106	98
2,4,5-Trichloroaniline	86	105	88

(a) 1 liter of spiked reagent H_2O (10 µg/L) was extracted 3 times with 60 mL CH_2Cl_2 using 2 minute shaking times and the extract was concentrated to 1.0 mL. Each extract was analyzed twice by HPLC.

(b) Estimation based on total area of the three unresolved peaks.

ments are presented in Table 17. As shown in this table, only the most basic anilines (e.g., aniline and the haloanilines) were extracted into 1 N H_2SO_4 from methylene chloride. Therefore, acid back extraction was not considered further in this program, although this procedure might be very effective when only aniline or monohaloanilines are to be determined.

Adsorption alumina, basic alumina, and Florisil were evaluated using a variety of elution schemes. Experimental details are given in Section 5. Adsorption alumina was found to give very poor recoveries (5-80%) for the anilines even with 100% diethyl ether as the elution solvent. Addition of trimethylamine (1%) to the diethyl ether eluent did not substantially improve recovery of the anilines. Basic alumina also gave poor recoveries (33-90%) for the anilines when using either the activated or methanol deactivated adsorbent. The elution characteristics for the various anilines from basic alumina are given in Table 18. Aniline recovery was less than 1% using this elution scheme and was only slightly higher (4%) using the methanol deactivation scheme.

The Florisil cleanup schemes described in Section 5 gave consistently better recoveries than did the various alumina cleanup schemes. However, the two activated Florisil schemes both gave poor recoveries for aniline (15-45%) and the dinitroanilines (<5-30%). The elution patterns for the anilines using the water deactivated and isopropanol deactivated Florisil elution schemes are given in Tables 19 and 20, respectively. Both deactivated Florisil systems gave better recovery than any of the other systems investigated for the anilines. The scheme utilizing isopropanol deactivated Florisil gave somewhat better recoveries, especially for aniline, than did the water deactivated system (compare Tables 19 and 20). The trichloroanilines were eluted more readily than the other anilines in both schemes.

On the basis of these results, the Florisil/5% isopropanol deactivation procedure (eliminating the 10% CH_2Cl_2 in hexane fraction which contained no anilines) was selected for use in the final method. However, the water deactivation procedure also appeared to be useful for aniline determination. The two procedures gave similar backgrounds for Wastewater 2a when analyzed by GC-NPD, as shown in Figure 7. The isopropanol deactivation procedure was favored because it eliminated the need to prepare adsorbent before hand and no fines were produced, as was the case with the H_2O deactivation procedure.

Storage Stability Evaluation

In the first phase of the storage stability study, six waters at two spike levels (20 and 200 μg/L) were stored at pH 2 (adjusted with sulfuric acid) and 4°C. These storage conditions were believed, on the basis of data obtained previously for benzidine (16), to represent the optimum conditions for preservation of the anilines. The wastewaters studied were reagent water, Columbus POTW effluent, Wastewater 3a, Wastewater 4a, Wastewater 4a after addition of 2 mg/L of sodium hypochlorite, and an industrial effluent from a coking operation (oven quench water). The last sample (coking effluent) was not relevant to the aniline methodology and was selected to represent an industrial effluent matrix without any aniline background contribution.

TABLE 17. DISTRIBUTION OF ANILINES BETWEEN CH_2Cl_2 AND 1N H_2SO_4

Compound	% Extracted Into 1N H_2SO_4[a]		
	Run A	Run B	Average
Aniline	102	106	104
4-Nitroaniline	18	19	18.5
3-Nitroaniline	80	83	81.5
2-Nitroaniline	2	1	1.5
2,4-Dinitroaniline	<1	<1	<1
4-Chloroaniline	99	108	104
3-Chloroaniline	89	91	90
2-Chloroaniline	51[b]	<1	51
2-Chloro-4-nitroaniline	<1	<1	<1
4-Bromoaniline	97[b]	<1	97
2-Chloro-4,6-dinitroaniline	<1	<1	<1
2-Bromo-4,6-dinitroaniline	1	2	1.5
4-Chloro-2-nitroaniline	<1	<1	<1
3,4-Dichloroaniline	58	58	58
2,6-Dichloro-4-nitroaniline	<1	<1	<1
2-Bromo-6-chloro-4-nitroaniline	<1	<1	<1
2,6-Dibromo-4-nitroaniline	<1	<1	<1
2,4,5-Trichloroaniline	<1	<1	<1

(a) Equal volumes of 1N sulfuric acid and methylene chloride containing 50 μg/mL of each aniline were equilibrated.

(b) Based on single determination of individual aniline compound.

TABLE 18. ELUTION OF ANILINES FROM BASIC ALUMINA USING HEXANE-DIETHYL ETHER-METHANOL ELUTION SCHEME WITH HEXANE COLUMN PACKING PROCEDURE [a]

Compound	% Recovery in Fraction [b]			
	50% diethyl ether in hexane (F3)	100% diethyl ether (F4)	10% methanol in diethyl ether (F5)	Total % Recovery
Aniline	<1	<1	<1	<1
4-Nitroaniline	<1	43	3	46
3-Nitroaniline	<1	37	15	52
2-Nitroaniline	70	12	<1	82
2,4-Dinitroaniline	<1	30	59	89
4-Chloroaniline	<1	5	30	35
3-Chloroaniline	<1	8	40	48
2-Chloroaniline	<1	<1	37	37
2-Chloro-4-nitroaniline	<1	<1	82	82
4-Bromoaniline	35	16	<1	51
2-Chloro-4,6-dinitroaniline	63	8	<1	71
2-Bromo-4,6-dinitroaniline	43	7	<1	50
4-Chloro-2-nitroaniline	74	10	<1	84
3,4-Dichloroaniline	<1	12	47	59
2,6-Dichloro-4-nitroaniline	30	3	<1	33
2-Bromo-6-chloro-4-nitroaniline	72	20	<1	92
2,6-Dibromo-4-nitroaniline	68	18	<1	86
2,4,5-Trichloroaniline	58	7	<1	65
2,4,6-Trichloroaniline	44	2	<1	46

(a) Basic alumina soxhlet extracted with methylene chloride and activated at 160°C for 16 hours before use. Twenty grams of adsorbent placed in chromatography column and rinsed with 80 mL of hexane before loading compounds of interest. Each eluent fraction was 50 mL volume.

(b) Fractions 1 and 2, hexane and 15% diethyl ether in hexane, respectively, did not contain detectable levels of aniline compounds.

TABLE 19. FLORISIL CLEANUP EVALUATION: 1% WATER DEACTIVATED FLORISIL[a]

	% Recovery in Fraction[b]			
Compound	50% CH_2Cl_2 in hexane (F3)	100% CH_2Cl_2 (F4)	5% Acetone in CH_2Cl_2 (F5)	Total % Recovery
Aniline	--	15	38	53
4-Nitroaniline	--	--	112	112
3-Nitroaniline	--	6	94	100
2-Nitroaniline	--	--	92	92
2,4-Dinitroaniline	--	9	90	99
4-Chloroaniline	--	70	2	72
3-Chloroaniline	--	86	2	88
2-Chloroaniline	--	77	2	79
2-Chloro-4-nitroaniline	--	3	93	96
4-Bromoaniline	--	66	--	66
2-Chloro-4,6-dinitroaniline	--	2	94	96
2-Bromo-4,6-dinitroaniline	--	--	99	99
4-Chloro-2-nitroaniline	--	47	45	92
3,4-Dichloroaniline	--	90	1	91
2,6-Dichloro-4-nitroaniline	--	51	26	77
2-Bromo-6-chloro-4-nitroaniline	--	64	34	98
2,6-Dibromo-4-nitroaniline	--	70	26	96
2,4,5-Trichloroaniline	2	86	--	88
2,4,6-Trichloroaniline	35	13	--	48

(a) Florisil extracted for 16 hours (Soxhlet) with CH_2Cl_2, activated for 16 hours at 160°C, 1% (by weight), H_2O added, and tumbled for 4 hours. Twenty grams of adsorbent placed in chromatographic column and rinsed with 80 mL of hexane prior to loading the compounds of interest. Fractions 1-4 were 50 mL volumes and Fraction 5 was 150 mL volume.

(b) Fractions 1 and 2, hexane and 10% CH_2Cl_2 in hexane, respectively, did not contain detectable levels of anilines.

TABLE 20. FLORISIL CLEANUP EVALUATION: 5% ISOPROPANOL (IPA) IN HEXANE DEACTIVATED FLORISIL

	% Recovery in Given Fraction[b]							
	50% CH_2Cl_2 in hexane		5% IPA in CH_2Cl_2		5% CH_3OH in CH_2Cl_2		Total Percent Recovery	
Compound	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Aniline	-	-	28	54	74	30	102	84
2-Chloroaniline	-	-	d		d		d	
3-Chloroaniline	-	-	81	75	5	2	86	77
4-Chloroaniline	0	14	53	59	22	5	75	64
4-Bromoaniline	-	-	71	70	16	5	87	75
3,4,-Dichloroaniline	-	-	93	73	0	2	93	75
2,4,6-Trichloroaniline[c]	51	54	17	11	-	-	86	84
2,4,5-Trichloroaniline	37	33	55	50	-	-	92	83
2-Nitroaniline	-	-	95	101	13	5	108	106
3-Nitroaniline	-	-	103	92	15	8	118	100
4-Nitroaniline	-	-	63	71	40	20	103	91
2,4-Dinitroaniline	-	-	-	-	73	78	73	78
4-Chloro-2-nitroaniline	-	-	90	78	3	0	93	78
2-Chloro-4-nitroaniline	-	-	d		d		d	
2,6-Dichloro-4-nitroaniline	-	-	92	87	10	9	102	96
2,6-Dibromo-4-nitroaniline	-	-	95	84	6	12	101	96
2-Bromo-6-chloro-4-nitroaniline	-	-	92	84	15	18	107	102
2-Chloro-4,6-dinitroaniline	-	-	-	-	75	77	75	77
2-Bromo-4,6-dinitroaniline	-	-	-	-	109	105	109	105

(a) Florisil extracted (Soxhlet) for 16 hours with CH_2Cl_2, then activated at 160°C for 16 hours prior to use. Twenty grams of activated Florisil placed in chromatographic column containing 80 mL of 5% isopropanol in hexane, then eluted with 50 mL of hexane prior to loading compounds. Fractions collected were as follows: 75 mL of hexane, 50 mL of 10% CH_2Cl_2 in hexane, 50 mL of 50% CH_2Cl_2 in hexane, 50 mL of 5% isopropanol in CH_2Cl_2, and 50 mL of 5% methanol in CH_2Cl_2.

(b) 5 µg of each aniline on column.

(c) 2,4,6-Trichloroaniline elutes partially in 10% CH_2Cl_2 in hexane fraction: 18% in Rep 1 and 19% in Rep 2.

(d) 2-Chloroaniline and 2-chloro-4-nitroaniline coelute with 4-bromoaniline. The percent recovery value for 4-bromoaniline is average value for all three compounds.

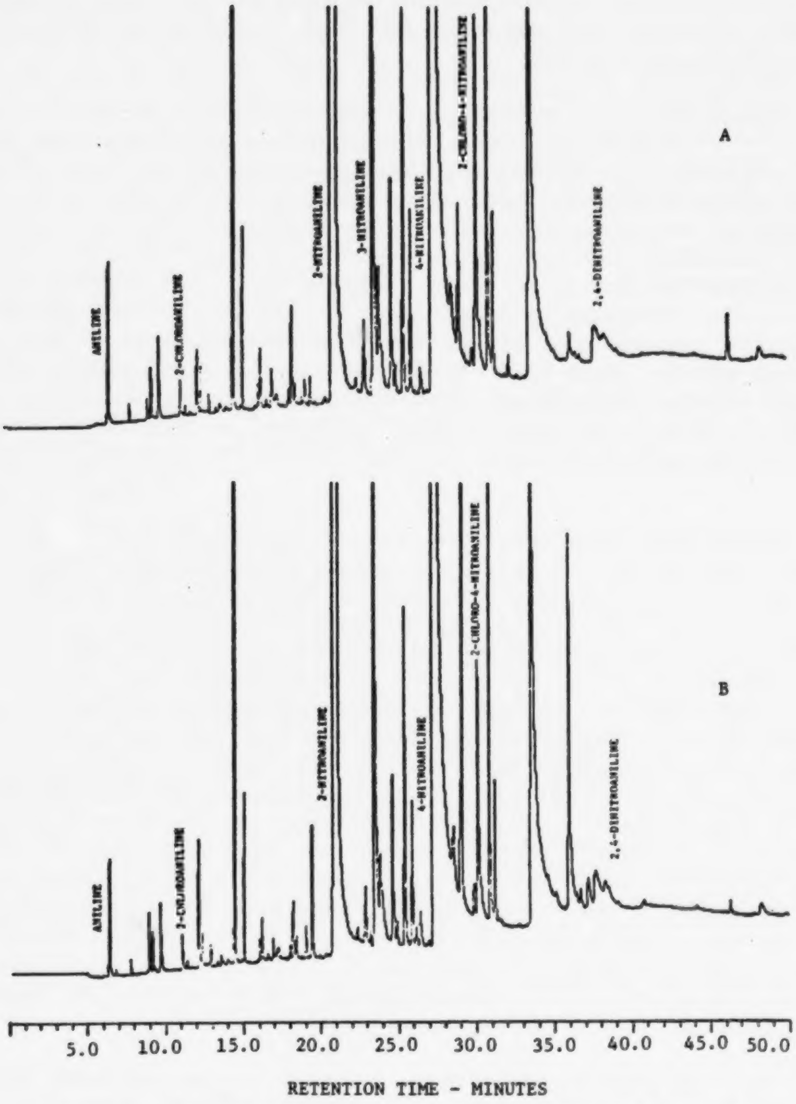

Figure 7. Comparison of the two Florisil cleanup techniques for Wastewater 2a using gas chromatography with fused silica SE-54 column and alkali flame detector.

A. 1% deactivated (H_2O) Florisil

B. 5% deactivated (isopropanol) Florisil.

Significant losses of certain anilines (especially aniline, 2-chloroaniline, 4-chloroaniline, and 4-bromoaniline) were observed in all samples at the 20 μg/L level. Even at the 200 μg/L level, significant losses were observed in two wastewaters (coking effluent and Wastewater 3a). In addition, the recovery of anilines in the 0-day samples was less than expected (30-50%) in many cases, especially for Wastewater 3a. Even reagent water gave a lower recovery than expected (50-70%), based on the previous extraction and cleanup recovery studies.

These data were surprising since extraction at neutral pH had been shown to give excellent recovery. Therefore, an experiment was conducted in which the anilines were spiked into Wastewater 3a at pH 2 and pH 6.5 and the sample extracted within one hour after spiking. Both samples were adjusted to pH 6.5 prior to extraction. The spike levels for the two samples were mistakenly different (20 μg/L at pH 2 and 50 μg/L at pH 6.5). However, it is obvious from the data (presented in Table 21), that spiking into the acidified sample resulted in significant losses for all of the anilines. Recoveries for the anilines spiked into the wastewater at pH 6.5 were considerably greater than 100%, presumably due to a systematic error in the analysis or spiking procedures. Serious attempts were not made to ascertain the source of this error since further experiments were to be conducted using the pH 6.5 level and any systematic errors could be ascertained from those data.

Additional experiments were conducted to evaluate the reasons for aniline loss at low pH. Purging the sample with nitrogen prior to spiking, addition of 20 mg/L of sodium thiosulfate, and addition of sodium hypophosphite all were ineffective in improving the recovery for anilines spiked into water at pH 2. On the basis of the results described above, pH 7 and 4°C were considered to be the optimum storage conditions. These conditions were evaluated using two spiked (20 μg/L) wastewaters and spiked reagent water. The storage stability data for Wastewater 4a, Wastewater 3a, and reagent water are presented in Tables 22, 23, and 24, respectively. Good stability was observed for most of the anilines in the two wastewaters although 10-15% losses of many of the anilines occurred over the seven-day storage period. However, overall recoveries were less than for distilled water. The anilines were found to be stable in non-chlorinated reagent water, but many of the compounds (e.g., aniline and some halogenated anilines) were unstable in the chlorinated sample, as shown in Table 24. The anilines lost to the greatest extent in the chlorinated reagent water are generally the most readily oxidized. This result appears to be reasonable based on the oxidizing capability of hypochlorite.

In a subsequent experiment, several of the anilines (especially the dinitroanilines) were found to be unstable (40-60% loss over a seven-day period) in a raw POTW influent (Columbus). The addition of 0.1% toluene, to retard microbial activity, did not improve the stability of these compounds and the reasons for loss of anilines in this sample are unknown. Irreversible adsorption onto suspended solids may have been a factor since the dinitroanilines tend to be more readily adsorbed than the other anilines and these compounds were lost to the largest extent.

TABLE 21. EFFECT OF INITIAL pH ON EXTRACTION EFFICIENCY OF ANILINES FROM WASTEWATER 3a

Compound	Percent Recovery[a] pH 2[b,c]	Percent Recovery[a] pH 6.5[c]
Aniline	<5	130
2-Chloroaniline	<5	130
3-Chloroaniline	7	120
4-Chloroaniline	10	130
4-Bromoaniline	<5	170
3,4-Dichloroaniline	<5	160
2,4,6-Trichloroaniline	<5	140
2,4,5-Trichloroaniline	<5	140
2-Nitroaniline	<5	140
3-Nitroaniline	<5	130
4-Nitroaniline	7	120
2,4-Dinitroaniline	9	99
4-Chloro-2-nitroaniline	6	120
2-Chloro-4-nitroaniline	<5	130
2,6-Dichloro-4-nitroaniline	10	94
2,6-Dibromo-4-nitroaniline	<5	110
2-Bromo-6-chloro-4-nitroaniline	32	180
2-Chloro-4,6-dinitroaniline	12	100
2-Bromo-4,6-dinitroaniline	7	98

(a) Average for duplicate analyses.
(b) pH adjusted to 6.5 prior to extraction.
(c) pH 2 samples spiked with 20 μg/L and pH 6.5 samples spiked with 50 μg/L of each aniline.

TABLE 22. RESULTS OF 0 and 7-DAY STORAGE STABILITY STUDY ON WASTEWATER 4a

Compound	Background Level, µg/L	Amount Spiked, µg/L	Amount Found in µg/L in 0-Day Sample Rep. 1	Rep. 2	$\bar{x}$	Average Percent Recovery	Amount Found in µg/L in 7-Day Sample Rep. 1	Rep. 2	$\bar{x}$	Average Percent Recovery
Aniline	--	20	12	10	11	57	9.8	8.8	9.3	47
2-Chloroaniline	--	20	20	18	19	93	17	16	16	83
3-Chloroaniline	--	20	15	13	14	69	13	11	12	61
4-Chloroaniline	--	20	11	9.6	10	53	10	9	10	49
4-Bromoaniline	--	20	11	10	10	50	10	7.8	8.9	45
3,4-Dichloroaniline	--	20	14	12	13	64	13	9	11	55
2,4,6-Trichloroaniline[a]	6.4	20	18	14	16	81	16	12	14	70
2,4,5-Trichloroaniline	--	20	17	14	16	79	16	13	14	72
2-Nitroaniline[a]	--	20	18	14	16	81	16	12	14	70
3-Nitroaniline	--	20	15	12	13	67	13	9.5	11	57
4-Nitroaniline[b]	0.62	20	16	13	14	72	14	10	12	60
2,4-Dinitroaniline	--	20	12	11	12	58	11	5	8	40
4-Chloro-2-nitroaniline[b]	--	20	16	13	14	72	14	10	12	60
2-Chloro-4-nitroaniline	--	20	18	15	16	81	15	12	14	68
2,6-Dichloro-4-nitroaniline	--	20	16	14	15	76	15	10	13	63
2,6-Dibromo-4-nitroaniline	--	20	22	18	20	98	8	12	10	51
2-Bromo-6-chloro-4-nitroaniline	--	20	20	17	19	94	18	13	16	78
2-Chloro-4,6-dinitroaniline	--	20	11	12	11	57	11	4.3	8	39
2-Bromo-4,6-dinitroaniline	--	20	11	12	11	57	10	4.3	7	36

(a) These compounds coelute. Equal response and recovery were assumed.
(b) These compounds coelute. Equal response and recovery were assumed.

TABLE 23. RESULTS OF 0 AND 7-DAY STORAGE STABILITY STUDY ON WASTEWATER 3a

Compound	Background Level, μg/L	Amount Spiked, μg/L	Amount Found in μg/L in 0-Day Sample Rep. 1	Percent Recovery	Amount Found in μg/L in 7-Day Sample Rep. 1	Rep. 2	$\bar{x}$	Average Percent Recovery
Aniline	1.7	20	14	72	11	15	13	65
2-Chloroaniline	7.6	20	8.3	42	5.6	9.5	7.6	38
3-Chloroaniline	--	20	16	79	12	17	14	70
4-Chloroaniline	--	20	6.9	35	11	15	13	65
4-Bromoaniline	1.3	20	15	75	11	18	15	75
3,4-Dichloroaniline	4.9	20	22	109	14	24	19	95
2,4,6-Trichloroaniline[a]	--	20	18	89	13	19	16	80
2,4,5-Trichloroaniline	--	20	19	93	12	19	16	80
2-Nitroaniline[a]	--	20	18	89	13	19	16	80
3-Nitroaniline	--	20	16	80	10	18	14	70
4-Nitroaniline[b]	--	20	18	88	9.5	18	14	70
2,4-Dinitroaniline	--	20	11	59	5.7	16	11	65
4-Chloro-2-nitroaniline[b]	--	20	18	88	9.5	18	14	70
2-Chloro-4-nitroaniline	--	20	20	101	11	20	16	80
2,6-Dichloro-4-nitroaniline	--	20	15	76	11	16	13	65
2,6-Dibromo-4-nitroaniline	--	20	13	66	8.1	15	12	60
2-Bromo-6-chloro-4-nitroaniline	9.4	20	28	142	13	36	24	120
2-Chloro-4,6-dinitroaniline	1.2	20	15	76	8.7	19	14	70
2-Bromo-4,6-dinitroaniline	--	20	13	66	7.5	17	12	60

(a) These compounds coelute. Equal response and recovery were assumed.

(b) These compounds coelute. Equal response and recovery were assumed.

TABLE 24. RESULTS OF 7-DAY STORAGE STABILITY STUDY ON CHLORINATED AND NON-CHLORINATED REAGENT WATER

Compound	Amount Spiked, µg/L	Amount Found in µg/L in Non-Chlorinated Samples			Average Percent Recovery	Amount Found in µg/L in Chlorinated Samples[a]			Average Percent Recovery
		Rep. 1	Rep. 2	$\bar{x}$		Rep. 1	Rep. 2	$\bar{x}$	
Aniline	20	14	12	13	65	--	--	--	0
2-Chloroaniline	20	17	14	16	79	--	0.8	0.4	2
3-Chloroaniline	20	15	13	14	70	--	--	--	0
4-Chloroaniline	20	15	12	14	68	--	--	--	0
4-Bromoaniline	20	15	13	14	70	--	--	--	0
3,4-Dichloroaniline	20	16	13	15	73	0.8	--	0.4	2
2,4,6-Trichloroaniline[b]	20	16	13	15	74	15	16	15	76
2,4,5-Trichloroaniline	20	17	14	15	76	4.8	--	2.4	12
2-Nitroaniline[b]	20	16	13	15	74	15	16	15	76
3-Nitroaniline	20	14	10	12	61	--	--	--	0
4-Nitroaniline[c]	20	14	10	12	60	7.6	10	8.8	44
2,4-Dinitroaniline	20	ND[d]	ND	ND	--	ND	ND	ND	--
4-Chloro-2-nitroaniline[c]	20	14	10	12	60	7.6	10	8.8	44
2-Chloro-4-nitroaniline	20	14	9.6	12	59	8.0	12	10	49
2,6-Dichloro-4-nitroaniline	20	18	13	16	78	11	12	12	58
2,6-Dibromo-4-nitroaniline	20	15	12	13	67	9	13	11	55
2-Bromo-6-chloro-4-nitroaniline	20	17	13	15	76	10	15	13	63
2-Chloro-4,6-dinitroaniline	20	14	10	12	62	11	18	14	72
2-Bromo-4,6-dinitroaniline	20	14	10	12	59	10	15	13	63

(a) Two mg/L of sodium hypochlorite added prior to spiking.
(b) These compounds coelute. Equal response and recovery were assumed.
(c) These compounds coelute. Equal response and recovery were assumed.
(d) Not determined due to poor column performance (peak was tailing severely).

On the basis of these results, storage of samples at pH 6-8 and 4°C, with added sodium thiosulfate to destroy active chlorine, was considered to be the best preservation approach for the anilines. In a subsequent experiment, the analysis of an extract of spiked Wastewater 4a was repeated after the extract had been stored for 36 days at 4°C. The results of this experiment are shown in Table 25. As shown by these results, the compounds were generally stable in the extract over this time period, although several compounds (2-chloro-, 3-chloro-, and 2-nitroaniline) gave higher values upon reanalysis, apparently due to some differences in instrument calibration. Only 3,4-dinitroaniline was significantly lower after storage of the extract for 36 days.

Validation of GC-NPD Method

Based on the results of the experiments described above, an analytical method was selected for validation. This method, described in detail in Appendix A, involves the following steps:

- Extraction of the wastewater at pH 11 with methylene chloride
- Concentration of the extract by Kuderna-Danish concentration
- Cleanup of the extract using Florisil deactivated with isopropanol
- Exchange of the final concentrate into toluene
- Analysis by GC-NPD.

Exchange of the extract into toluene was necessary in order to obtain good column performance using splitless injection onto capillary columns. As described earlier, ethylene dichloride was used as the injection solvent for GC-MS work, but could not be used with GC-NPD systems. All of the validation work described below involved the use of an SE-54 FSCC. Florisil Fractions 1, 2, and 3 were combined prior to concentration and analysis (see Appendix A for details).

Method Detection Limits--

Method detection limits (MDL) for the anilines were determined for reagent water, Wastewater 3a, Wastewater 4a, and POTW (Columbus) effluent using the U.S. EPA/EMSL-Cincinnati procedure given in Appendix B. The MDL data for these samples are given in Tables 26-29. As shown in these tables, the MDL values were similar for all the samples and ranged between 1 and 12 µg/L for the various compounds. 4-Nitroaniline and 2,4-dinitroaniline gave the poorest MDL values in most cases. Recoveries were generally greater than 60%, except for aniline (29%), 4-chloroaniline (7.3%), and 4-bromoaniline (23%) in the reagent water. The percent relative standard deviations were generally 20% or less. Several compounds had high positive bias (>100% recovery) in Wastewater 3a. In all cases, these compounds were also present in the nonspiked wastewater, leading to the possible explanation that the true background level may have been greater than was observed in the single analysis, thus resulting in a positive bias in the spike recovery data.

TABLE 25. RESULTS OF REANALYSIS OF STORED EXTRACT FROM WASTEWATER 4a

Compound	Spike Level µg/L	Amount Found in µg/L Original Analysis	Amount Found in µg/L Reanalysis after 36-day storage
Aniline	20	17	20
2-Chloroaniline	20	9.3	15
3-Chloroaniline	20	8.0	12
4-Chloroaniline	20	7.3	9.5
4-Bromoaniline	20	7.2	8.9
3,4-Dichloroaniline	20	8.5	9.3
2,4,6-Trichloroaniline	20	7.4	11
2,4,5-Trichloroaniline	20	8.5	11
2-Nitroaniline	20	3.7	7.2
3-Nitroaniline	20	8.6	9.9
4-Nitroaniline	20	10	11
3,4-Dinitroaniline	20	20	11
4-Chloro-2-Nitroaniline	20	10	11
2-Chloro-4-Nitroaniline	20	13	13
2,6-Dichloro-4-Nitroaniline	20	11	13
2,6-Dibromo-4-Nitroaniline	20	11	12
2-Bromo-6-Chloro-4-Nitroaniline	20	16	17
2-Chloro-4,6-Dinitroaniline	20	15	13
2-Bromo-4,6-Dinitroaniline	20	15	13

TABLE 26. RESULTS OF METHOD DETECTION LIMIT STUDY FOR ANILINE COMPOUNDS IN REAGENT WATER

Compound	Amount Spiked, µg/L	Amount found in µg/L in Given Spiked Replicate										Average Percent Recovery
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Average	SD [a]	MDL [b]	
Aniline	8.0	1.7	2.3	2.4	2.5	3.2	3.1	1.1	2.3	0.73	2.3	29
2-Chloroaniline	4.0	3.2	3.6	3.6	3.1	3.8	3.5	2.5	3.4	0.44	1.4	84
3-Chloroaniline	4.0	2.0	2.4	1.9	1.9	2.0	2.1	0.64	1.9	0.57	1.8	47
4-Chloroaniline	4.0	0.22	0.22	0.056	0.47	0.67	0.27	0.11	0.29	0.21	0.66 [c]	7.3
4-Bromoaniline	16	4.1	3.4	5.1	3.9	4.7	4.3	0.59	3.7	1.5	4.6	23
3,4-Dichloroaniline	8.0	3.1	3.6	3.7	3.6	4.9	3.8	1.5	3.5	1.0	3.2	43
2,4,6-Trichloroaniline	8.0	5.6	7.5	5.3	7.6	7.6	8.6	11	7.6	1.9	5.8	95
2,4,5-Trichloroaniline	8.0	6.1	6.3	7.4	6.9	9.0	7.4	6.7	7.1	0.95	3.0	89
2-Nitroaniline	4.0	3.6	4.0	3.9	3.1	3.3	3.3	3.6	3.5	0.33	1.0	88
3-Nitroaniline	8.0	3.7	4.5	4.9	4.7	6.2	4.6	2.8	4.5	1.0	3.3	56
4-Nitroaniline	8.0	4.3	11	3.5	8.8	6.5	6.8	13.1	7.7	3.4	11	96
2,4-Dinitroaniline	16	11	13	16	12	19	15	13	14	2.8	8.9	89
4-Chloro-2-nitroaniline	8.0	6.9	8.1	8.0	6.0	6.1	6.6	6.5	6.9	0.85	2.7	86
2-Chloro-4-nitroaniline	8.0	6.3	6.5	8.1	6.9	9.3	7.4	7.3	7.4	1.0	3.2	92
2,6-Dichloro-4-nitroaniline	8.0	6.2	6.3	7.7	6.5	8.8	7.2	7.1	7.1	0.93	2.9	89
2,6-Dibromo-4-nitroaniline	8.0	7.4	7.5	9.4	7.3	10	8.8	7.6	8.3	1.2	3.8	100
2-Bromo-6-chloro-4-nitroaniline	8.0	7.4	7.5	9.2	7.6	10	8.4	8.2	8.4	1.1	3.4	100
2-Chloro-4,6-dinitroaniline	8.0	5.5	6.1	6.9	4.6	8.3	6.5	6.0	6.2	1.2	3.6	78
2-Bromo-4,6-dinitroaniline	8.0	5.4	6.0	6.8	4.4	8.1	6.3	5.6	6.1	1.2	3.7	76

(a) SD = Standard Deviation
(b) MDL = Method Detection Limit
(c) Due to the low recoveries obtained for this compound, the actual MDL is expected to be approximately equal to the spike level.

TABLE 27. RESULTS OF METHOD DETECTION LIMIT STUDY FOR ANILINE COMPOUNDS IN WASTEWATER 3a

Compound	Amount Spiked, µg/L	Background Level, µg/L	Amount Found in µg/L in Given Spiked Replicate										
			Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Rep. 6	Rep. 7	Average	SD[a]	MDL[b]	Average Percent Recovery
Aniline	10	0.17	12	15	15	11	10	13	12	13	1.8	5.6	130
2-Chloroaniline	5.0	--	4.0	5.2	5.1	4.5	3.7	4.4	4.6	4.5	0.53	1.7	90
3-Chloroaniline	5.0	0.76	3.7	4.8	5.0	4.0	3.4	4.1	4.3	4.2	0.56	1.8	83
4-Chloroaniline	5.0	--	4.1	5.1	5.2	4.3	3.8	4.4	4.6	4.5	0.51	1.6	90
4-Bromoaniline	20	1.8	26	35	30	27	24	24	28	28	4.0	13	140
3,4-Dichloroaniline	10	7.0	10	15	16	10	8.9	13	12	12	2.6	8.2	120
2,4,6-Trichloroaniline[c]	10	--	9.0	11	12	9.5	8.3	9.6	10	9.9	1.2	3.8	99
2,4,5-Trichloroaniline	10	--	8.0	9.9	11	9.0	7.8	9.0	9.9	9.2	1.1	3.6	92
2-Nitroaniline[c]	5.0	--	4.5	5.3	6.0	4.8	4.1	4.8	5.1	5.0	0.61	1.9	99
3-Nitroaniline	10	--	9.5	12	14	11	9.8	11	12	11	1.5	4.6	110
4-Nitroaniline[d]	10	1.3	12	16	20	15	12	15	15	15	2.8	8.7	150
2,4-Dinitroaniline	20	--	13	13	22	17	16	18	22	17	3.7	12	87
4-Chloro-2-nitroaniline[d]	10	--	12	16	20	15	12	15	15	15	2.8	8.7	150
2-Chloro-4-nitroaniline	10	--	11	13	18	12	12	13	13	13	2.1	6.5	130
2,6-Dichloro-4-nitroaniline	10	--	11	11	14	12	10	12	13	12	1.2	3.8	120
2,6-Dibromo-4-nitroaniline	10	--	7.4	10	11	8.7	8.4	9.8	10	9.4	1.3	4.2	94
2-Bromo-6-chloro-4-nitroaniline	10	--	12	15	19	15	14	16	17	15	2.1	6.6	150
2-Chloro-4,6-dinitroaniline	10	--	9.1	11	16	12	10	12	14	12	2.3	7.2	120
2-Bromo-4,6-dinitroaniline	10	--	4.4	4.7	6.8	5.7	5.0	5.6	6.3	5.5	0.85	2.7	55

(a) SD = Standard Deviation.
(b) MDL = Method Detection Limit.
(c) These two compounds coelute. Equal response and recovery were assumed.
(d) These two compounds coelute. Equal response and recovery were assumed.

TABLE 28. RESULTS OF METHOD DETECTION LIMIT STUDY FOR ANILINE COMPOUNDS IN WASTEWATER 4a

Compound	Amount Spiked, μg/L	Background Level, μg/L	Amount Found in μg/L in Given Spiked Replicate							Average	SD[a]	MDL[b]	Average Percent Recovery
			Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Rep. 6	Rep. 7				
Aniline	10	--	6.2	4.6	5.2	5.8	5.8	2.8	5.8	5.2	1.2	3.7	52
2-Chloroaniline	5.0	--	5.6	4.2	5.0	4.6	5.0	5.0	5.4	5.0	0.47	1.5	99
3-Chloroaniline	5.0	--	4.6	2.8	3.6	3.2	3.6	3.4	4.0	3.6	0.58	1.8	72
4-Chloroaniline	5.0	--	3.6	2.4	3.0	3.0	3.0	2.8	3.4	3.0	0.39	1.2	61
4-Bromoaniline	20	--	16	10	14	13	14	12	15	14	1.8	5.8	68
3,4-Dichloroaniline	10	--	8.0	4.6	7.2	7.6	7.2	6.8	8.4	7.1	1.2	3.9	71
2,4,6-Trichloroaniline[c]	10	6.0	11	4.8	9.9	11	12	8.9	15	10	3.1	9.7	100
2,4,5-Trichloroaniline	10	--	8.6	5.4	8.4	8.2	8.0	4.0	9.6	7.5	2.0	6.2	75
2-Nitroaniline[c]	5.0	--	5.4	2.4	4.9	5.4	5.7	4.4	7.6	5.1	1.6	4.9	100
3-Nitroaniline	10	0.37	8.2	6.0	7.6	7.8	8.0	8.0	9.0	7.8	0.91	2.9	78
4-Nitroaniline[d]	10	0.61	8.8	6.4	8.4	8.2	8.4	4.0	9.2	7.6	1.8	5.8	76
2,4-Dinitroaniline	20	--	18	16	20	17	21	15	21	18	2.5	7.9	91
4-Chloro-2-nitroaniline[d]	10	--	8.8	6.4	8.4	8.2	8.4	4.0	9.2	7.6	1.8	5.8	76
2-Chloro-4-nitroaniline	10	0.62	9.4	7.4	8.6	8.8	9.6	13	11	9.7	1.9	6.1	97
2,6-Dichloro-4-nitroaniline	10	--	8.6	5.4	8.0	7.6	7.0	8.8	9.0	7.8	1.3	4.0	78
2,6-Dibromo-4-nitroaniline	10	--	9.4	6.8	9.8	9.0	9.2	8.8	9.4	8.9	0.99	3.1	89
2-Bromo-6-chloro-4-nitroaniline	10	--	10	7.2	10	10	9.8	10	10	9.8	1.2	3.7	98
2-Chloro-4,6-dinitroaniline	10	--	8.2	6.2	8.6	6.8	8.2	11	8.4	8.2	1.6	5.0	82
2-Bromo-4,6-dinitroaniline	10	--	7.4	5.8	8.4	7.4	7.8	8.6	7.8	7.6	0.92	2.9	76

(a) SD = Standard Deviation.
(b) MDL = Method Detection Limit.
(c) These two compounds coelute. Equal recovery was assumed.
(d) These two compounds coelute. Equal response and recovery were assumed.

TABLE 29. RESULTS OF METHOD DETECTION LIMIT STUDY FOR ANILINE COMPOUNDS IN COLUMBUS POTW EFFLUENT

Compound	Amount Spiked, µg/L	Background Level, µg/L	Amount Found in µg/L in Given Spiked Replicate									
			Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Rep. 6	Average[a]	SD[b]	MDL[c]	Average Percent Recovery
Aniline	10	(d)	6.8	5.6	6.3	6.8	7.2	6.9	6.6	0.59	2.0	66
2-Chloroaniline	5.0	(d)	4.1	3.5	4.0	4.7	4.7	4.6	4.3	0.48	1.6	85
3-Chloroaniline	5.0	(d)	3.5	2.8	3.3	3.9	3.9	3.9	3.6	0.44	1.5	71
4-Chloroaniline	5.0	(d)	3.4	2.8	3.4	3.6	3.9	3.8	3.5	0.38	1.3	70
4-Bromoaniline	20	(d)	10	9.0	11	12	13	13	11	1.6	5.5	57
3,4-Dichloroaniline[e]	10	(d)	5.4	4.5	5.9	7.1	7.0	6.9	6.1	1.0	3.5	61
2,4,6-Trichloroaniline	10	(d)	7.7	6.0	7.4	9.6	9.1	8.7	8.1	1.3	4.4	81
2,4,5-Trichloroaniline	10	(d)	6.2	4.7	6.1	8.0	7.6	7.2	6.6	1.2	4.1	66
2-Nitroaniline[e]	5.0	(d)	3.9	3.0	3.7	4.8	4.5	4.4	4.0	0.65	2.2	81
3-Nitroaniline	10	(d)	5.0	4.1	5.3	6.6	6.9	6.3	5.7	1.1	3.6	57
4-Nitroaniline[f]	10	(d)	5.2	4.1	5.5	7.2	7.1	6.3	5.9	1.2	4.1	59
2,4-Dinitroaniline	20	(d)	8.4	5.3	7.7	14	14	10	9.9	3.5	12	50
4-Chloro-2-nitroaniline[f]	10	(d)	5.2	4.1	5.5	7.2	7.1	6.3	5.9	1.2	4.1	59
2-Chloro-4-nitroaniline	10	(d)	5.6	4.3	5.8	8.3	7.8	7.0	6.5	1.5	5.1	65
2,6-Dichloro-4-nitroaniline	10	(d)	5.8	4.3	5.9	8.5	7.9	7.1	6.6	1.5	5.1	66
2,6-Dibromo-4-nitroaniline	10	(d)	5.1	3.8	5.2	7.8	7.3	6.5	6.0	1.5	5.1	60
2-Bromo-6-chloro-4-nitroaniline	10	(d)	6.3	4.6	6.4	9.5	8.8	7.7	7.2	1.8	6.1	72
2-Chloro-4,6-dinitroaniline	10	(d)	4.9	3.3	4.7	7.4	7.1	5.8	5.5	1.6	5.3	55
2-Bromo-4,6-dinitroaniline	10	(d)	4.5	3.0	4.5	7.2	7.0	5.5	5.3	1.6	5.5	53

(a) Average of six instead of seven samples.
(b) SD = Standard Deviation.
(c) MDL = Method Detection Limit.
(d) No significant background.
(e) These two compounds coelute. Equal response and recovery were assumed.
(f) These two compounds coelute. Equal response and recovery were assumed.

Representative chromatograms, using the SE-54 FSCC, for Wastewaters 3a and 4a are given in Figures 8 and 9, respectively. A chromatogram using the packed 3% SP-2250 column (specified as an alternate column in the method) for Wastewater 4a is shown in Figure 10. The superior resolution of the anilines from background components using capillary columns is clearly demonstrated by these chromatograms.

Analytical Curve--

The analytical curves for the various anilines were determined using the method described in Appendix C. All steps in the method, including Florisil cleanup, were utilized in this study. The analytical curve data are given in Table 30. Linear least squares regression analysis results are given in Table 31. Recoveries for the majority of the anilines were constant over the range tested (approximately 3-300 x MDL). However, poor recoveries at less than 10 x MDL were observed for aniline (29%), 4-chloroaniline (7%), and 4-bromoaniline (23%). Nevertheless, all of the anilines were readily detectable at the lowest spike level. The compounds giving the lowest recoveries are those most readily oxidized. Hence, oxidation during the workup procedures may explain some of the losses observed at low spike levels.

High Spike Level Validation--

The performance of the method at higher spike levels was evaluated for reagent water, Wastewater 3a, and Wastewater 4a. Spike levels were approximately 100 μg/L (20-100 x MDL) in all cases. The results for this evaluation are given in Tables 32, 33, and 34. Recoveries were generally 70-120% for all samples, except for aniline (55%) and 4-chloroaniline (49%) in distilled water. Percent relative standard deviations were generally 5-15%.

The MDL, analytical curve, and high level spike data indicate that the method, given in Appendix A, can be used to determine all of the aniline compounds with good precision and accuracy in a variety of matrices. However, very low recoveries are frequently found for aniline, 4-chloroaniline, and 4-bromoaniline at concentrations approaching the MDL, as previously discussed.

GC-MS (U.S. EPA Method 625) Evaluation

The tasks included in this segment of the study were: 1) the acquisition of mass spectra for the 19 anilines, and 2) the evaluation, modification, and validation of U.S. EPA Method 625 for the determination of anilines in wastewater. Both capillary and packed column approaches were evaluated using relevant wastewaters as well as reagent water.

Acquisition of Mass Spectra--

Initially, mass spectra were collected from material eluted from both packed and capillary columns, using the instrument parameters given in Section 5. When available, library spectra were compared to the spectra obtained using both packed and capillary columns. Spectra for all 19 anilines are included in Appendix D. The spectra shown were acquired using packed column GC-MS, except for aniline which was acquired using capillary column since the packed column spectrum contained a small amount of an alkyl benzene

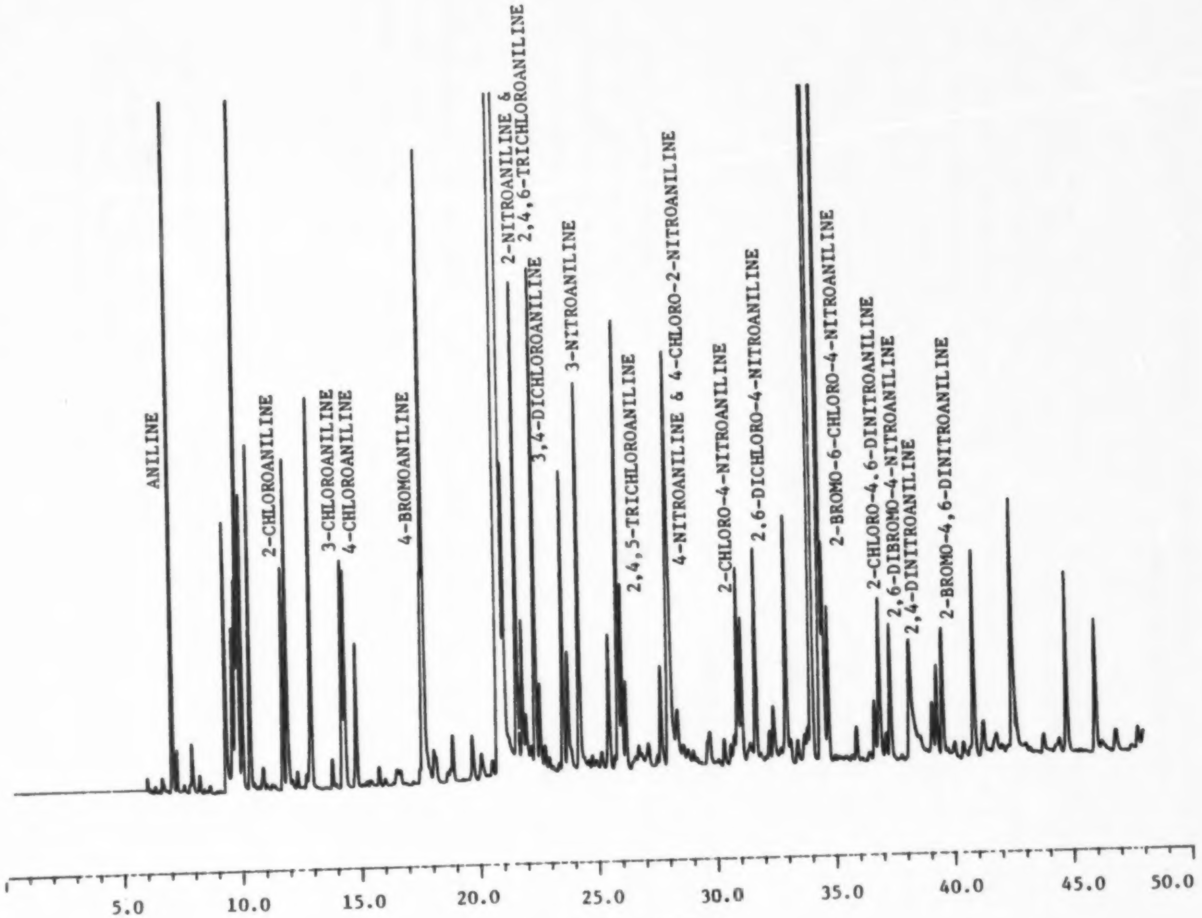

Figure 8. GC-NPD chromatogram for spiked Wastewater 3a using an SE-54 fused silica capillary column. See Table 27 for spike level.

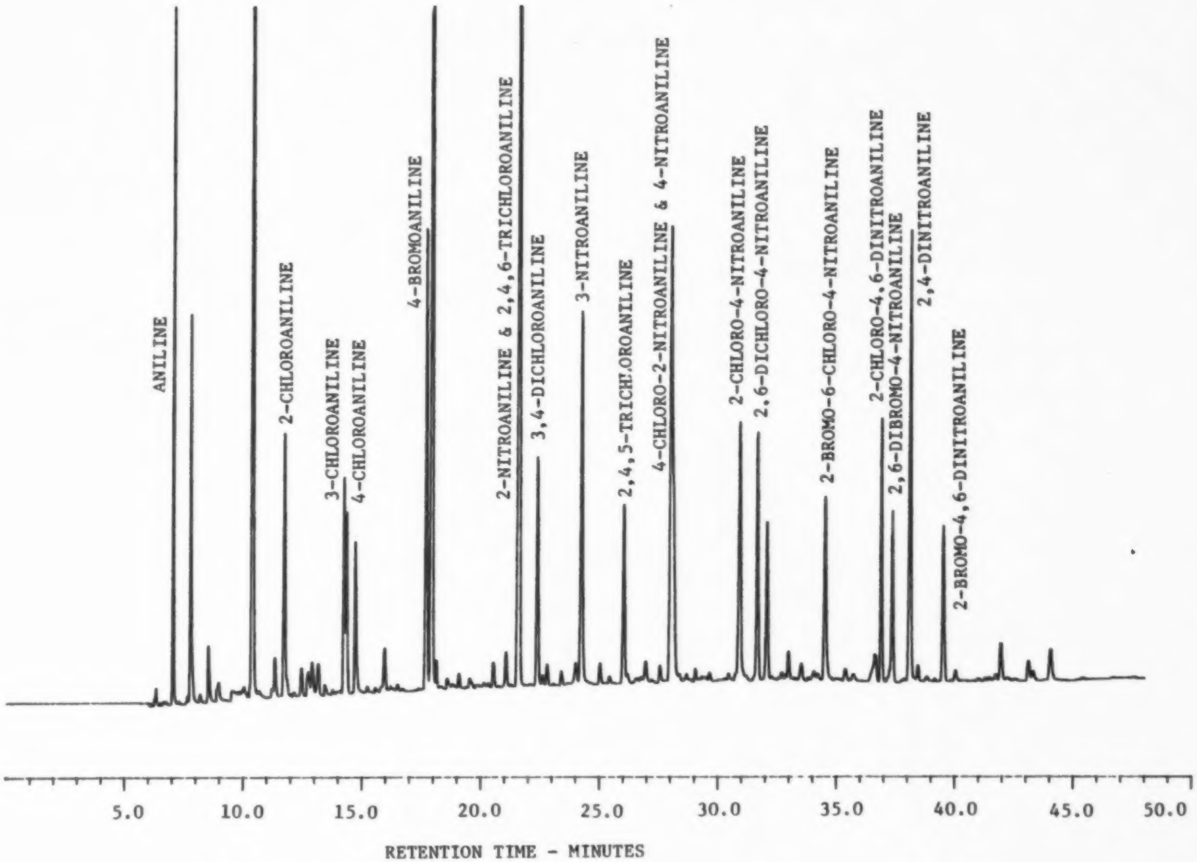

Figure 9. GC-NPD chromatogram for spiked Wastewater 4a using an SE-54 fused silica capillary column. See Table 28 for spike level.

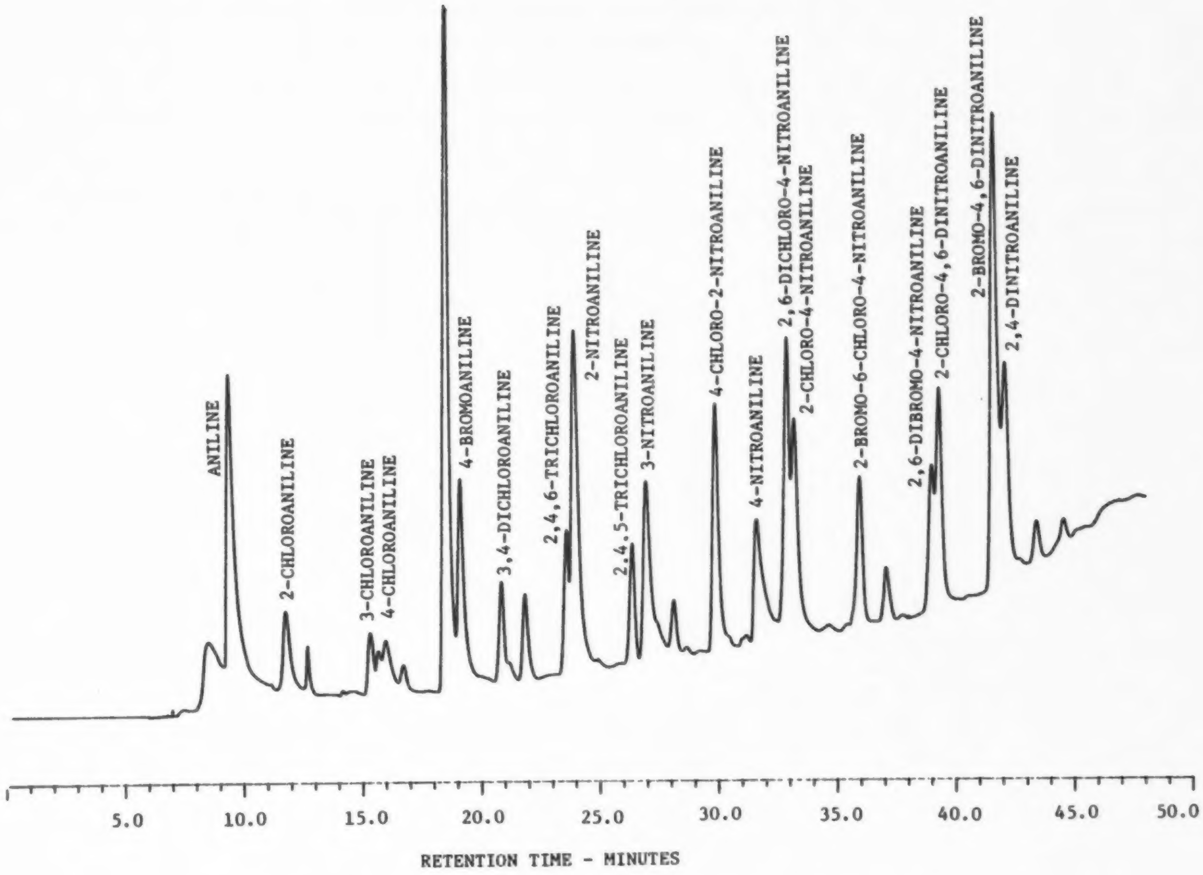

Figure 10. GC-NPD chromatogram for Wastewater 4a using a 3% SP-2250 packed column. See Table 28 for spike level.

TABLE 30. ANALYTICAL CURVE DATA FOR ANILINE, 2-CHLOROANILINE, 3-CHLOROANILINE AND 4-CHLOROANILINE IN REAGENT WATER

Aniline			2-Chloroaniline			3-Chloroaniline			4-Chloroaniline		
Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery
8.0	2.3 [a]	29	4.0	3.4 [a]	84	4.0	1.9 [a]	47	4.0	0.3 [a]	7.3
40	18	46	20	14	72	20	13	64	20	3.3	17
40	18	44	20	15	74	20	14	75	20	3.2	16
80	44 [a]	55	40	31 [a]	78	40	32 [a]	80	40	19 [a]	49
400	360	90	200	190	94	200	180	90	200	140	68
400	310	76	200	170	83	200	160	82	200	120	60
800	680	86	400	360	91	400	360	91	400	290	73
800	600	74	400	310	78	400	300	75	400	330	84

(a) Average of seven replicates from validation study. All other data represent individual analyses.

TABLE 30. ANALYTICAL CURVE DATA FOR 4-BROMOANILINE, 3,4-DICHLOROANILINE, 2,4,6-TRICHLOROANILINE AND 2,4,5-TRICHLOROANILINE IN REAGENT WATER (CONTINUED)

4-Bromoaniline			3,4-Dichloroaniline			2,4,6-Trichloroaniline			2,4,5-Trichloroaniline		
Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery
16.0	3.7 [a]	23	8.0	3.5 [a]	43	8.0	7.6 [a]	95	8.0	7.1 [a]	89
80	41	52	40	22	56	40	39	97	40	27	67
80	44	55	40	22	56	40	36	90	40	28	70
160	110 [a]	67	80	56 [a]	70	80	68 [a]	85	80	63 [a]	79
800	760	95	400	380	95	400	360	91	400	380	95
800	700	88	400	360	91	400	320	80	400	340	84
1600	1600	100	800	810	100	800	650	81	800	780	98
1600	1300	81	800	650	81	800	600	76	800	780	98

(a) Average of seven replicates from validation study. All other data represent individual analyses.

TABLE 30. ANALYTICAL CURVE DATA FOR 2-NITROANILINE, 3-NITROANILINE, 4-NITROANILINE, AND 2,4-DINITROANILINE IN REAGENT WATER (CONTINUED)

2-Nitroaniline			3-Nitroaniline			4-Nitroaniline			2,4-Dinitroaniline		
Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery
4.0	3.5 [a]	88	8.0	4.5 [a]	56	8.0	7.7 [a]	96	16	14 [a]	89
20	15	76	40	19	47	40	31	77	80	51	64
20	16	79	40	19	49	40	33	82	80	48	60
40	35 [a]	88	80	53 [a]	66	80	60 [a]	75	160	120 [a]	74
200	260	130	400	180	45	400	480	120	800	760	95
200	250	125	400	170	43	400	480	120	800	800	100
400	380	94	800	780	97	800	1100	133	1600	1800	113
400	300	75	800	560	69	800	1000	130	1600	1500	94

(a) Average of seven replicates from validation study. All other data represent individual analyses.

TABLE 30. ANALYTICAL CURVE DATA FOR 4-CHLORO-2-NITROANILINE, 2-CHLORO-4-NITROANILINE, 2,6-DICHLORO-4-NITROANILINE, AND 2,6-DIBROMO-4-NITROANILINE IN REAGENT WATER (CONTINUED)

4-Chloro-2-nitroaniline			2-Chloro-4-nitroaniline			2,6-Dichloro-4-nitroaniline			2,6-Dibromo-4-nitroaniline		
Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery
8.0	6.9 [a]	86	8.0	7.4 [a]	92	8.0	7.1 [a]	89	8.0	8.3 [a]	100
40	33	82	40	27	68	40	27	67	40	27	68
40	34	84	40	27	68	40	28	69	40	28	70
80	73 [a]	91	80	64 [a]	80	80	66 [a]	82	80	68 [a]	85
400	390	98	400	380	94	400	380	95	400	340	85
400	380	96	400	360	91	400	360	89	400	330	83
800	860	110	800	850	110	800	840	110	800	780	97
800	630	79	800	780	98	800	780	98	800	680	86

(a) Average of seven replicates from validation study. All other data represent individual analyses.

TABLE 30. ANALYTICAL CURVE DATA FOR 2-BROMO-6-CHLORO-4-NITROANILINE, 2-CHLORO-4,6-DINITROANILINE, AND 2-BROMO-4,6-DINITROANILINE IN REAGENT WATER (CONTINUED)

2-Bromo-6-chloro-4-nitroaniline			2-Chloro-4,6-dinitroaniline			2-Bromo-4,6-dinitroaniline		
Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery	Amount Spiked, µg/L	Amount Found, µg/L	Percent Recovery
8.0	8.4 [a]	100	8.0	6.2 [a]	78	8.0	6.1 [a]	76
40	31	77	40	23	57	40	22	55
40	32	81	40	25	63	40	24	60
80	76 [a]	95	80	62 [a]	78	80	62 [a]	77
400	420	100	400	370	93	400	370	92
400	400	100	400	360	91	400	370	92
800	940	120	800	750	94	800	860	110
800	860	110	800	860	110	800	740	92

(a) Average of seven replicates from validation study. All other data represent individual analyses.

TABLE 31. LINEAR LEAST SQUARES PARAMETERS FOR ANALYTICAL CURVE DATA [a]

Compound	Slope [b]	Y-Intercept	Correlation Coefficient
Aniline	0.82	-11	0.9993
2-Chloroaniline	0.97	- 6.2	0.9991
3-Chloroaniline	0.84	- 1.1	0.9998
4-Chloroaniline	0.79	-12	0.9967
4-Bromoaniline	0.93	-26	0.9998
3,4-Dichloroaniline	0.93	-10	0.9997
2,4,6-Trichloroaniline	0.79	7.3	0.9992
2,4,5-Trichloroaniline	0.98	-13	0.9992
2-Nitroaniline	0.90	10	0.9712
3-Nitroaniline	0.80	-29	0.9713
4-Nitroaniline	1.3	-26	0.9991
2,4-Dinitroaniline	1.0	-31	0.9996
4-Chloro-2-nitroaniline	0.94	- 0.21	0.9997
2-Chloro-4-nitroaniline	1.0	-15	0.9990
2,6-Dichloro-4-nitroaniline	1.0	-15	0.9988
2,6-Dibromo-4-nitroaniline	0.91	- 8.0	0.9991
2-Bromo-6-chloro-4-nitroaniline	1.1	-14	0.9991
2-Chloro-4,6-dinitroaniline	1.0	-16	0.9992
2-Bromo-4,6-dinitroaniline	1.0	-16	0.9993

(a) Calculated from average values from Table 30.
(b) X-axis = Expected (spiked) concentration, µg/L
Y-axis = Found concentrations, µg/L

TABLE 32. RESULTS OF METHOD VALIDATION STUDY FOR ANILINE COMPOUNDS IN REAGENT WATER

Compound	Amount Spiked, µg/L	Amount Found in µg/L in Given Spiked Replicate										Average Percent Recovery
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Average	SD a	%RSD b	
Aniline	80	48	48	46	46	42	40	40	44	3.5	7.9	55
2-Chloroaniline	40	35	33	34	32	29	28	27	31	3.3	11	78
3-Chloroaniline	40	37	33	34	34	29	28	27	32	3.7	12	80
4-Chloroaniline	40	20	24	18	21	15	18	19	19	2.9	15	49
4-Bromoaniline	160	130	110	110	115	98	96	94	107	12	11	67
3,4-Dichloroaniline	80	70	56	58	60	50	49	47	56	7.8	14	70
2,4,6-Trichloroaniline	80	65	81	60	71	62	74	64	68	7.5	11	85
2,4,5-Trichloroaniline	80	80	64	66	66	59	56	52	63	8.9	14	79
2-Nitroaniline	40	43	36	37	37	32	31	30	35	4.7	13	88
3-Nitroaniline	80	66	52	55	57	50	47	44	53	7.2	14	66
4-Nitroaniline	80	58	85	44	70	49	61	54	60	14	23	75
2,4-Dinitroaniline	160	160	110	110	130	130	110	93	120	21	17	74
4-Chloro-2-nitroaniline	80	92	71	76	76	70	64	60	73	10	14	91
2-Chloro-4-nitroaniline	80	82	62	66	68	63	57	51	64	9.7	15	80
2,6-Dichloro-4-nitroaniline	80	84	64	68	69	64	59	54	66	9.7	15	82
2,6-Dibromo-4-nitroaniline	80	88	66	66	72	68	61	55	68	10	15	85
2-Bromo-6-chloro-4-nitroaniline	80	97	73	76	79	76	68	62	76	11	15	95
2-Chloro-4,6-dinitroaniline	80	82	56	60	68	64	56	49	62	10	17	78
2-Bromo-4,6-dinitroaniline	80	82	55	56	68	65	56	49	62	11	18	77

(a) SD = Standard Deviation
(b) %RSD = percent relative standard deviation

TABLE 33. RESULTS OF METHOD VALIDATION STUDY FOR ANILINE COMPOUNDS IN WASTEWATER 3a

Compound	Back-ground Level, µg/L	Amount Spiked, µg/L	Amount Found in µg/L in Given Spiked Replicate										Average Percent Recovery
			Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Average	SD [a]	%RSD [b]	
Aniline	0.17	100	78	75	74	81	78	82	77	78	3.0	3.9	78
2-Chloroaniline	—	100	84	81	83	89	84	89	82	85	3.3	3.8	85
3-Chloroaniline	0.76	100	80	78	81	89	84	88	80	83	4.3	5.2	83
4-Chloroaniline	—	100	80	78	80	90	86	89	82	84	4.8	5.8	84
4-Bromoaniline	1.8	100	87	87	90	104	99	103	93	95	7.3	7.7	95
3,4-Dichloroaniline	7.0	100	85	84	91	102	96	99	90	92	6.9	7.5	92
2,4,6-Trichloroaniline [c]	—	100	86	85	92	99	92	98	86	91	5.7	6.2	91
2,4,5-Trichloroaniline	—	100	86	83	92	98	93	97	86	91	6.1	6.7	91
2-Nitroaniline [c]	—	100	86	84	92	99	92	98	86	91	5.7	6.2	91
3-Nitroaniline	—	100	88	86	95	108	102	106	96	97	8.5	8.8	97
4-Nitroaniline [d]	1.3	100	100	130	110	96	98	100	93	100	12	11	100
2,4-Dinitroaniline	—	100	98	95	120	160	130	140	150	130	27	21	130
4-Chloro-2-nitroaniline [d]	—	100	100	130	110	96	98	100	93	100	12	11	100
2-Chloro-4-nitroaniline	—	100	91	86	96	120	100	110	110	100	12	12	100
2,6-Dichloro-4-nitroaniline	—	100	94	89	100	120	100	110	100	100	9.3	9.2	100
2,6-Dibromo-4-nitroaniline	—	100	91	82	88	120	98	100	100	97	11	12	97
2-Bromo-6-chloro-4-nitroaniline	—	100	120	110	120	150	130	140	140	130	15	12	130
2-Chloro-4,6-dinitroaniline	—	100	98	90	100	140	120	130	130	120	19	16	120
2-Bromo-4,6-dinitroaniline	—	100	98	89	110	140	120	130	130	120	19	17	120

(a) SD = Standard Deviation
(b) %RSD = percent relative standard deviation
(c) These two compounds coelute; equal response and recovery were assumed
(d) These two compounds coelute; equal response and recovery were assumed.

TABLE 34. RESULTS OF METHOD VALIDATION STUDY FOR ANILINE COMPOUNDS IN WASTEWATER 4a

Compound	Background Level, µg/L	Amount Spiked, µg/L	Amount Found in µg/L in Given Spiked Replicate										Average Percent Recovery
			Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Average	SD[a]	%RSD[b]	
Aniline	---	100	90	84	77	74	88	68	74	79	8.2	10	79
2-Chloroaniline	---	100	110	110	96	89	110	84	91	98	11	11	98
3-Chloroaniline	---	100	100	100	90	84	110	78	86	92	11	12	92
4-Chloroaniline	---	100	99	96	88	83	100	76	84	90	10	11	90
4-Bromoaniline	---	100	100	100	92	88	110	80	90	96	11	11	96
3,4-Dichloroaniline	---	100	100	100	94	90	120	81	94	98	12	13	98
2,4,6-Trichloroaniline[c]	6.0	100	110	110	99	93	120	83	99	100	13	13	100
2,4,5-Trichloroaniline	---	100	100	110	96	91	120	82	98	100	12	12	100
2-Nitroaniline[c]		100	110	110	99	93	120	83	99	100	13	13	100
3-Nitroaniline	0.37	100	97	100	91	91	120	81	100	98	14	14	98
4-Nitroaniline[d]	0.61	100	96	98	90	92	130	82	100	99	15	15	99
2,4-Dinitroaniline	---	100	110	120	98	130	190	120	170	130	32	24	130
4-Chloro-2-nitroaniline[d]		100	96	98	90	92	130	82	100	99	15	15	99
2-Chloro-4-nitroaniline	0.63	100	110	110	100	100	140	95	120	110	17	15	110
2,6-Dichloro-4-nitroaniline	---	100	110	110	100	100	140	91	110	110	14	13	110
2,6-Dibromo-4-nitroaniline	---	100	98	98	91	98	130	90	110	100	14	14	100
2-Bromo-6-chloro-4-nitroaniline	---	100	120	120	110	120	160	100	130	120	17	14	120
2-Chloro-4,6-dinitroaniline	---	100	100	100	96	110	150	100	130	110	18	16	110
2-Bromo-4,6-dinitroaniline	---	100	110	100	95	110	150	100	130	110	19	16	110

(a) SD = Standard Deviation
(b) %RSD = percent relative standard deviation
(c) These two compounds coelute; equal response and recovery were assumed
(d) These two compounds coelute; equal response and recovery were assumed.

pattern (m/e 105, 77 cluster). The spectrum for 2,4,6-trichloroaniline also was acquired using capillary column since this compound was not evaluated during the initial phase of the program when packed column GC-MS was in use.

Good agreement was obtained between the spectra of eluate from capillary and packed column in most cases. However, the spectra for 2-nitro-substituted anilines eluted from packed columns contained a significantly greater M-18 (loss of water) fragment compared to the library or capillary column spectra. One hypothesis to explain this phenomenon is that a catalytic dehydration between the ortho nitro group and the amine group occurs in the transfer line of the mass spectrometer. This transfer line contained metal bulkhead connections at each end of the glass jet separator and a glass lined stainless steel tube about 15 cm long. Perhaps the metallic surface at 250°C catalyzes this reaction. The capillary column transfer line was fused silica with no exposed metal surfaces being present.

All the nitrosubstituted anilines showed somewhat enhanced M-16 and M-30 ions (loss of O and NO, respectively) relative to the library spectra using both packed and capillary column. The effect was usually more pronounced using packed column, again indicating a transfer line effect.

Based on the data obtained, a set of search and quantification ions for each compound was selected. The ions selected are shown in Table 35. The search criteria employed for qualitative identification were those specified in U.S. EPA Method 625, which are given below:

- Characteristic ions for each compound of interest were maximized in the same scan or within one scan of each other
- The retention time must fall within ± 30 seconds of the retention time of the authentic compound
- The relative peak heights of the three characteristic ions in the extracted ion current profile (EICP) must fall within ± 20% of the relative intensities of the ions in a reference spectrum.

In this study, all reference spectra were acquired using the GC-MS conditions employed for samples, because of the differences between library and GC-MS spectra noted above. In general, library spectra should not be used because the differences in instrumentation can greatly affect relative ion intensities. The selection of search ions for the various anilines (Table 35) was conducted according to the following guidelines.

- In general, the molecular ion was included as one of the search ions. In addition, the molecular ion was normally used as the quantification ion. However, for the three bromosubstituted compounds (2,6-dibromo-4-nitroaniline, 2-bromo-6-chloro-4-nitroaniline, and 2-bromo-4,6-dinitroaniline), the M-30 ion was used for

TABLE 35. EXTRACTED ION CURRENT PROFILE (EICP) SEARCH AND QUANTIFICATION IONS FOR THE 19 ANILINE COMPOUNDS

Compound	EICP Search And Quantification Ions [a], m/e		
Aniline	93	66[b]	
2-Chloroaniline	100	127	129
3-Chloroaniline	100	127	129
4-Chloroaniline	100	127	129
4-Bromoaniline	92	171	173
3,4-Dichloroaniline	90	161	163
2,4,6-Trichloroaniline	124	195	197
2,4,5-Trichloroaniline	124	195	197
2-Nitroaniline	92	108	138
3-Nitroaniline	92	108	138
4-Nitroaniline	80	108	138
2,4-Dinitroaniline	107	153	183
4-Chloro-2-nitroaniline	90	126	172
2-Chloro-4-nitroaniline	90	142	172
2,6-Dichloro-4-nitroaniline	124	176	206
2,6-Dibromo-4-nitroaniline	168	266	296
2-Bromo-6-chloro-4-nitroaniline	220	222	252
2-Chloro-4,6-dinitroaniline	128	187	217
2-Bromo-4,6-dinitroaniline	231	261	263

(a) The single ion used for quantification is underlined.
(b) In complex mixtures, m/e 66 may not be highly specific for aniline and GC retention time may be preferred confirmatory evidence.

quantification since this ion was the base peak and had a mass closer to that of the internal standard, D_{10}-anthracene. Quantification will be more precise for a given compound when the quantification ion is close to the internal standard quantification ion since relative intensity will be less affected by instrument tuning parameters.

- Whenever possible, two halogen isotope ions were included in the search list, since the ratios of these two ions should be stable and specific for a given compound.
- Low mass ions (e.g., m/e 70 or less) were excluded except for aniline itself, due to a high degree of interference from other compounds in this mass region.
- The M-18 peak was excluded due to the transfer line effect on ion intensity discussed above. The M-30 peak was generally included only for those compounds where the relative intensities of this ion was greater than 50% for both packed and capillary column, the only exception being 3-nitroaniline.
- Search ions selected were evaluated for interferences using a spiked POTW extract, and only those ions exhibiting relatively low background interference were included.

As indicated in Table 35, the molecular ion may be the only definitive search ion for aniline. Although m/e 66 is included in the ion search list for aniline, it may not be useful since there was a high degree of interference in this region, which gave rise to a high degree of variability in the relative abundance for this ion. On the basis of this study, the use of a single search ion (m/e 93) for aniline appears to be best in complex mixtures, since m/e 93 is in a mass range less susceptible to the usually encountered interferences. Other than $C_6H_7N^+$ (aniline), $C_6H_5O^+$ ion is the only formula for m/e 93 which might present significant interferences.

Evaluation of U.S. EPA Method 625--

U.S. EPA Method 625 (base neutral extraction) was evaluated using both the packed and capillary column conditions described in Section 5. The 3% SP-2250 packed column specified in U.S. EPA Method 625 and a SE-54 FSCC (J&W Scientific) were used in these studies. Experimental procedures were exactly as given in U.S. EPA Method 625 except that the final extract was exchanged into ethylene dichloride and the packed column was treated with BSTFA injections before use. No significant mass spectral differences were noted for any of the compounds between the FSCC and the glass capillary column used to acquire reference spectra. In all cases, search criteria were defined based on mass spectra generated on the GC-MS system used for the sample analyses.

Results for replicate analysis of three spiked wastewaters on both packed and capillary column are shown in Tables 36-41. A 25 µg/L spike level was used in all cases, except for the capillary column analysis of POTW wastewater (Table 37) which was spiked at lower levels in order to approach the limit of detection using capillary column. Performance data for the method using reagent water is shown in Table 42. Recoveries for reagent water, Columbus POTW wastewater, and Wastewater 3a were generally greater than 60%, except for the packed column analysis of Columbus POTW wastewater which gave 40-60% recoveries for several compounds. The low spike levels are probably a contributing factor to the low recoveries observed. The >100% recoveries observed are probably due to the relative large amount of analyte in the background sample. In retrospect, higher spike levels probably should have been investigated as well.

Wastewater 2a had a high level of aniline and other compounds, especially nitroaromatics, which made analysis of the sample more difficult. Certain compounds (2,4-dinitroaniline and 2,6-dichloro-4-nitroaniline) could not be determined at the 25 µg/L spike level packed column because of a high degree of background interference. The dinitro-substituted anilines could not be determined by capillary column because the column performance deteriorated, resulting in highly asymmetric peaks. The GC-NPD method given in Appendix A gives suitable provisions for assessing capillary column performance prior to use in the method. This approach should also be used when determining anilines (especially nitroanilines) using U.S. EPA Method 625. Whether or not the column deterioration was related to this particular sample is unknown, since these samples were run on the same day as a number of other samples. Reanalysis of the sample was not feasible because the supply was exhausted. The aniline value for Wastewater 2a obtained using packed column is not correct because the quantification ion was saturated.

Good agreement between capillary and packed column data was obtained for Wastewater 3a (Tables 40 and 41), where no substantial interferences were observed and the same extracts were analyzed by both techniques. In general, higher recoveries were observed for Columbus POTW wastewater using capillary column. However, extracts analyzed by the two techniques were different, since a lower spike level for capillary column analysis was desired. Therefore, the difference observed for this sample may be due to extraction efficiency differences rather than column performance.

On the other hand, somewhat lower recoveries were observed for Wastewater 2a for many of the compounds using capillary column compared to packed column. However, the higher relative standard deviation for packed column results compared to capillary column results and the large quantities of interferences in this sample make it difficult to assess whether or not the two data sets are significantly different.

Detection limits were estimated using data from both packed and capillary columns for analyses of Wastewaters 2a and 3a using U.S. EPA Method 625. These data are given in Table 43. The data for Wastewater 3a

TABLE 36. RESULTS OF U.S. EPA METHOD 625 EVALUATION OF POTW WASTEWATER (CHARLES CITY) SPIKED AT 25 µg/L BY PACKED COLUMN GC-MS

Compound	Amount Found in Unspiked Sample, µg/L	Amount Found in Given Spiked Replicate, µg/L					Average Percent Recovery[b]
		Rep 1	Rep 2	Rep 3	Average	SD[a]	
Aniline	2.3	7.3	8.8	9.6	8.6	1.2	25
2-Chloroaniline	ND[d]	9.2	12	11	11	1.4	44
3-Chloroaniline[c]	ND	10	13	12	12	1.5	48
4-Chloroaniline[c]	ND	10	13	12	12	1.5	48
4-Bromoaniline	ND	11	14	11	12	1.7	48
3,4-Dichloroaniline	ND	13	17	13	14	2.3	56
2,4,6-Trichloroaniline	ND	11	15	12	13	2.1	52
2,4,5-Trichloroaniline	ND	9.9	14	12	12	2.1	48
2-Nitroaniline	6.9	27	37	27	30	5.8	92
3-Nitroaniline	ND	12	15	11	13	2.1	52
4-Nitroaniline	8.1	32	42	29	34	6.8	104
2,4-Dinitroaniline	ND	14	21	11	15	5.1	60
4-Chloro-2-nitroaniline	ND	10	13	8.8	11	2.2	44
2-Chloro-4-nitroaniline	ND	12	16	10	13	3.1	52
2,6-Dichloro-4-nitroaniline	ND	13	17	13	14	2.3	56
2,6-Dibromo-4-nitroaniline	ND	13	20	13	15	4.0	60
2-Bromo-6-chloro-4-nitroaniline	ND	13	18	11	14	3.6	56
2-Chloro-4,6-dinitroaniline	ND	12	19	9.4	13	5.0	52
2-Bromo-4,6-dinitroaniline	ND	12	19	8.6	13	5.3	52

(a) SD = Standard deviation.
(b) Corrected for amount found in unspiked sample.
(c) These compounds are not chromatographically resolved. The values shown are based upon one-half of the total response for the two isomers.
(d) Not detected

TABLE 37. RESULTS OF U.S. EPA METHOD 625 EVALUATION OF SPIKED POTW WASTEWATER (CHARLES CITY) BY CAPILLARY COLUMN GC-MS

Compound	Amount Spiked, µg/L	Amount Found in Unspiked Sample, µg/L	Amount Found in Given Spiked Replicate, µg/L						Average Percent Recovery[b]
			Rep 1	Rep 2	Rep 3	Rep 4	Average	SD[a]	
Aniline	12.5	0.8	7.9	11	9.8	11	9.8	1.5	72
2-Chloroaniline	5	ND[d]	3.7	4.7	4.0	4.2	4.2	0.42	84
3-Chloroaniline[c]	5	ND	3.4	4.4	3.7	3.9	3.9	0.42	78
4-Chloroaniline[c]	5	0.8	3.4	4.4	3.7	3.9	3.9	0.42	62
4-Bromoaniline	5	ND	3.8	4.9	4.2	4.4	4.3	0.46	86
3,4-Dichloroaniline	5	ND	4.2	5.0	4.4	4.8	4.6	0.37	92
2,4,6-Trichloroaniline	5	ND	3.5	4.2	3.5	3.7	3.7	0.33	74
2,4,5-Trichloroaniline	5	ND	3.7	4.4	3.7	4.0	4.0	0.33	80
2-Nitroaniline	5	4.6	14	17	14	13	15	1.4	208
3-Nitroaniline	12.5	ND	10	12	10	11	11	1.0	88
4-Nitroaniline	25	ND	18	23	22	23	22	2.9	88
2,4-Dinitroaniline	25	ND	9.8	15	15	18	14	3.4	56
4-Chloro-2-nitroaniline	12.5	ND	8.0	10	7.5	8.3	8.5	1.1	68
2-Chloro-4-nitroaniline	12.5	ND	8.6	11	8.7	9.3	9.4	1.1	75
2,6-Dichloro-4-nitroaniline	12.5	ND	8.9	11	8.5	9.0	9.4	1.1	75
2,6-Dibromo-4-nitroaniline	12.5	ND	7.8	10	9.2	9.8	9.2	1.0	74
2-Bromo-6-chloro-4-nitroaniline	12.5	ND	8.4	11	9.1	9.7	9.6	1.1	77
2-Chloro-4,6-dinitroaniline	25	ND	11	17	15	17	15	2.8	60
2-Bromo-4,6-dinitroaniline	25	ND	9.5	15	14	18	14	3.6	56

(a) SD = Standard deviation.
(b) Corrected for amount found in unspiked samp.
(c) These compounds are not chromatographically resolved. The values shown are based upon one-half of the total response of the two isomers.
(d) Not detected

TABLE 38. RESULTS OF U.S. EPA METHOD 625 EVALUATION OF WASTEWATER 2a SPIKED AT 25 µg/L BY PACKED COLUMN GC-MS

Compound	Amount Found in Unspiked Sample, µg/L	Amount Found in Given Spiked Replicate, µg/L Rep 1	Rep 2	Rep 3	Average	SD[a]	Average Percent Recovery[b]
Aniline	c	4200 d	3900 d	2700 d	3600	760	–
2-Chloroaniline	10	69	60	43	57	13	160
3-Chloroaniline(e)	2.5	33	31	21	28	6.4	100
4-Chloroaniline(e)	2.5	33	31	21	28	6.4	100
4-Bromoaniline	ND[g]	27	20	14	20	6.5	80
3,4-Dichloroaniline	ND	25	22	22	23	1.7	92
2,4,6-Trichloroaniline	ND	18	17	12	16	3.2	64
2,4,5-Trichloroaniline	ND	20	18	12	17	4.2	68
2-Nitroaniline	ND	26	21	18	22	4.0	88
3-Nitroaniline	ND	27	22	15	21	6.0	84
4-Nitroaniline	6.6	13	47	36	32	17	100
2,4-Dinitroaniline [f]	ND	—	—	—	—	—	<5
4-Chloro-2-nitroaniline	ND	25	20	13	19	6.0	76
2-Chloro-4-nitroaniline	ND	21	18	15	18	3.0	72
2,6-Dichloro-4-nitroaniline [f]	ND	—	—	—	—	—	<5
2,6-Dibromo-4-nitroaniline	ND	29	21	13	21	8.0	84
2-Bromo-6-chloro-4-nitroaniline	ND	30	23	17	23	6.5	84
2-Chloro-4,6-dinitroaniline	ND	32	23	17	24	7.5	96
2-Bromo-4,6-dinitroaniline [f]	ND	—	—	—	—	—	<5

(a) SD = Standard deviation.
(b) Corrected for amount found in unspiked sample.
(c) Amount not determined because of extremely high level.
(d) Quantification incorrect due to saturated peak.
(e) These compounds are not chromatographically resolved. The values shown are based upon one-half of the total response for the two isomers.
(f) High level of interference in the sample.
(g) Not detected.

TABLE 39. RESULTS OF U.S. EPA METHOD 625 EVALUATION OF WASTEWATER 2a SPIKED AT 25 µg/L BY CAPILLARY COLUMN GC-MS

Compound	Amount Found in Unspiked Sample, µg/L	Amount Found in Given Spiked Replicate, µg/L					Average Percent Recovery[b]
		Rep 1	Rep 2	Rep 3	Average	SD[a]	
Aniline	c	11,000[d]	8,400[d]	7,200[d]	8,900	1900	--
2-Chloroaniline	10	36	46	33	38	6.8	110
3-Chloroaniline[e]	2.5	20	22	16	19	3.1	73
4-Chloroaniline[e]	2.5	20	22	16	19	3.1	73
4-Bromoaniline	ND	11	14	10	12	2.1	48
3,4-Dichloroaniline	ND	13	14	10	12	2.1	48
2,4,6-Trichloroaniline	ND	13	13	10	8.7	5.1	35
2,4,5-Trichloroaniline	ND	14	13	10	12	2.1	48
2-Nitroaniline	ND	24	26	20	23	3.1	92
3-Nitroaniline	ND	13	14	8.2	12	3.1	48
4-Nitroaniline	6.4	52	42	31	42	11	130
2,4-Dinitroaniline[f]	ND	—	—	—	—	—	<5
4-Chloro-2-nitroaniline	ND	8.0	12	7.1	9.0	2.6	36
2-Chloro-4-nitroaniline	ND	18	20	15	18	2.5	72
2,6-Dichloro-4-nitroaniline	ND	16	16	12	15	2.3	60
2,6-Dibromo-4-nitroaniline[f]	ND	—	—	—	—	—	<5
2-Bromo-6-chloro-4-nitroaniline	ND	17	18	13	16	2.6	64
2-Chloro-4,6-dinitroaniline[f]	ND	—	—	—	—	—	<5
2-Bromo-4,6-dinitroaniline[f]	ND	—	—	—	—	—	<5

(a) SD = Standard deviation.
(b) Corrected for amount found in unspiked sample. See spiked sample data for aniline.
(c) Amount not determined due to extremely high level.
(d) Quantification after 625-fold dilution of sample.
(e) These compounds are not chromatographically resolved. The values shown are based upon one-half of the total response for the two isomers.
(f) Not detected due to poor column performance. These peaks were tailing very badly, giving rise to very high detection limits (see text).

TABLE 60. RESULTS OF U.S. EPA METHOD 625 EVALUATION OF WASTEWATER 3a SPIKED AT 25 µg/L BY PACKED COLUMN GC-MS

Compound	Amount Found in Unspiked Sample, µg/L	Amount Found in Given Spiked Replicate, µg/L					Average Percent Recovery
		Rep 1	Rep 2	Rep 3	Average	SD[a]	
Aniline	10	22	28	27	26	3.2	63 [b]
2-Chloroaniline	ND	16	17	15	15	1.5	60
3-Chloroaniline [c]	ND	16	19	18	17	2.6	68
4-Chloroaniline [c]	ND	16	19	18	17	2.6	68
4-Bromoaniline	ND	16	21	19	19	2.5	76
2,4-Dichloroaniline	ND	17	20	17	18	1.7	72
2,4,6-Trichloroaniline	ND	15	18	16	16	1.5	64
2,4,5-Trichloroaniline	ND	13	17	15	15	2.0	60
2-Nitroaniline	ND	17	21	19	19	2.0	76
3-Nitroaniline	ND	18	24	21	21	3.0	84
4-Nitroaniline	ND	12	19	20	17	4.4	68
2,4-Dinitroaniline	ND	19	32	28	26	6.7	100
4-Chloro-2-nitroaniline	ND	14	18	16	16	2.0	64
2-Chloro-4-nitroaniline	ND	16	19	18	18	1.5	72
2,6-Dichloro-4-nitroaniline	ND	16	19	16	17	1.7	68
2,6-Dibromo-4-nitroaniline	ND	18	24	21	21	3.0	84
2-Bromo-6-chloro-4-nitroaniline	ND	17	21	19	19	2.0	76
2-Chloro-4,6-dinitroaniline	ND	15	22	19	19	2.6	76
2-Bromo-4,6-dinitroaniline	ND	16	22	20	19	4.2	76

(a) SD = Standard deviation.
(b) These compounds are not chromatographically resolved. The values shown are based upon one-half of the total response for the two isomers.

TABLE 41. RESULTS OF U.S. EPA METHOD 625 EVALUATION OF WASTEWATER 3a SPIKED AT 25 µg/L BY CAPILLARY COLUMN GC-MS.

Compound	Amount Found in Unspiked Sample, µg/L	Amount Found in Given Spiked Replicate, µg/L					Average Percent Recovery
		Rep 1	Rep 2	Rep 3	Average	SD[a]	
Aniline	12	20	26	24	23	3.1	45
2-Chloroaniline	ND	13	16	13	14	1.7	56
3-Chloroaniline[b]	ND	14	17	15	15	1.5	60
4-Chloroaniline[b]	ND	14	17	15	15	1.5	60
4-Bromoaniline	ND	16	19	17	17	1.5	68
3,4-Dichloroaniline	ND	16	18	18	17	1.2	68
2,4,6-Trichloroaniline	ND	16	17	16	16	0.58	64
2,4,5-Trichloroaniline	ND	14	16	14	15	1.2	60
2-Nitroaniline	ND	14	16	15	15	1.0	60
3-Nitroaniline	ND	15	18	17	17	1.5	68
4-Nitroaniline	ND	15	18	17	17	1.5	68
2,4-Dinitroaniline	ND	22	18	19	20	2.1	80
4-Chloro-2-nitroaniline	ND	15	16	14	15	1.0	60
2-Chloro-4-nitroaniline	ND	15	16	15	15	0.58	60
2,6-Dichloro-4-nitroaniline	ND	15	17	16	16	1.0	64
2,6-Dibromo-4-nitroaniline	ND	16	19	17	17	1.5	68
2-Bromo-6-chloro-4-nitroaniline	ND	16	20	17	18	2.1	72
2-Chloro-4,6-dinitroaniline	ND	18	19	17	18	1.0	72
2-Bromo-4,6-dinitroaniline	ND	20	20	20	20	0.0	80

(a) SD = Standard deviation.

(b) These compounds are not chromatographically resolved. The values shown are based upon one-half of the total response for the two isomers.

TABLE 42. RESULTS OF CAPILLARY GC-MS ANALYSIS OF 10 μg/L SPIKED DISTILLED WATER EXTRACTS[a]

Compound	Average Percent Recovery	Standard Deviation
Aniline	55	14
2-Chloroaniline	63	14
3-Chloroaniline [b]	61	13
4-Chloroaniline [b]	61	13
4-Bromoaniline	68	14
2,4,6-Trichloroaniline	68	13
2-Nitroaniline	69	12
3,4-Dichloroaniline	74	13
2,4,5-Trichloroaniline	73	11
3-Nitroaniline	77	15
4-Chloro-2-nitroaniline	62	7
4-Nitroaniline	59	5
2,6-Dichloro-4-nitroaniline	74	7
2-Chloro-4-nitroaniline	70	6
2,6-Dibromo-4-nitroaniline	76	3
2-Bromo-6-chloro-4-nitroaniline	76	5
2-Chloro-4,6-dinitroaniline	72	3
2-Bromo-4,6-dinitroaniline	74	2
2,4-Dinitroaniline	78	2

(a) Data obtained for three one-liter aliquots.

(b) Not separated chromatographically. Equal contribution from the two isomers is assumed.

TABLE 43. ESTIMATED DETECTION LIMITS (DL) FOR WASTEWATERS 2a AND 3a BY GC ON PACKED AND CAPILLARY COLUMNS[a]

	Wastewater 2a		Wastewater 3a	
Compound	Packed	Capillary	Packed	Capillary
Aniline	b	b	4[c]	0.2
2-Chloroaniline	1	1	2	0.3
3-Chloroaniline	2	1	4	0.5
4-Chloroaniline	2	1	4	0.5
4-Bromoaniline	4	1	3	0.5
3,4-Dichloroaniline	6	3	3	0.5
2,4,6-Trichloroaniline	1	1	1	0.4
2,4,5-Trichloroaniline	1	0.5	0.5	0.4
2-Nitroaniline	5	2	5	3
3-Nitroaniline	18	8	8	2
4-Nitroaniline	7	25	8	2
2,4-Dinitroaniline	d	e	10	4
4-Chloro-2-nitroaniline	14	40	2	2
2-Chloro-4-nitroaniline	14	16	4	2
2,6-Dichloro-4-nitroaniline	d	8	4	2
2,6-Dibromo-4-nitroaniline	3	e	2	4
2-Bromo-6-chloro-4-nitroaniline	8	12	10	3
2-Chloro-4,6-dinitroaniline	6	e	4	4
2-Bromo-4,6-dinitroaniline	6	e	8	8

(a) Determined from peak height for quantification ion and a 5:1 signal to noise ratio.

(b) Saturated signal.

(c) All values µg/liter.

(d) Large interference. DL greater than 25 µg/L.

(e) Column performance deteriorated for these compounds. DL greater than 25 µg/L.

are the same as for distilled water, since no significant background interferences were observed in this sample. As shown in Table 43, the estimated detection limits for packed and capillary column are generally very comparable, although in most cases the capillary column values are somewhat better (notable exceptions being several nitro- and dinitro-substituted compounds in Wastewater 2a). Estimated detection limits of 10 µg/L or less were obtained for all the anilines in the low level sample (Wastewater 3a). Reconstructed ion chromatograms for Wastewater 3a using packed and capillary column are shown in Figure 11.

On the basis of these data, it appears that the basic U.S. EPA Method 625 approach is acceptable for the determination of anilines in a variety of wastewaters, using the minor modifications described earlier. The use of either packed or capillary columns is feasible. Both have certain advantages and disadvantages.

FSCCs have the advantage that the transfer line, which can adsorb anilines if not carefully deactivated, is eliminated. Capillary columns also resolve anilines from potential interferences better than packed column (see Figure 11) and give lower detection limits. However, capillary columns are more readily overloaded by high level components and require a somewhat higher degree of skill to manipulate. For example, splitless injection must be carried out at an appropriate temperature using a solvent with an appropriate boiling point in order to get adequate performance. This study has found ethylene dichloride or toluene injection solvents and an initial column temperature of 50°C to be suitable. As noted earlier, ethylene dichloride was chosen for GC-MS work, but was replaced by toluene for GC-NPD method since the NPD cannot accept chlorinated solvents. The poor performance initially observed for the most polar nitroanilines can apparently be ascribed to an insufficient liquidity of the SE-54 gum stationary phase at injection temperatures below 50°C. Adequate performance for 2,4-dinitroaniline must be verified as described in Appendix A (Section 12) whenever capillary GC-MS is performed.

Packed columns appear to work satisfactorily. However, the injection of replicate aliquots of silylating reagent (e.g., BSTFA) onto the system (with the outlet vented away from the MS inlet) is a requirement to achieve good peak shape (especially for aniline) at low levels.

SLUDGE METHOD EVALUATION

The objective of this phase of the program was to evaluate the U.S. EPA/EMSL-Cincinnati Sludge Method (3), originally developed for the determination of priority pollutants, for the aniline compounds. This method is presented in Appendix E. The work conducted in this study included an initial evaluation of the various steps of the method in terms of aniline recovery, optimization of the method parameters for aniline determination, determination of the partition ratio between liquid and solid phases for the anilines in sludge, and validation of the sludge protocol for the 19 anilines.

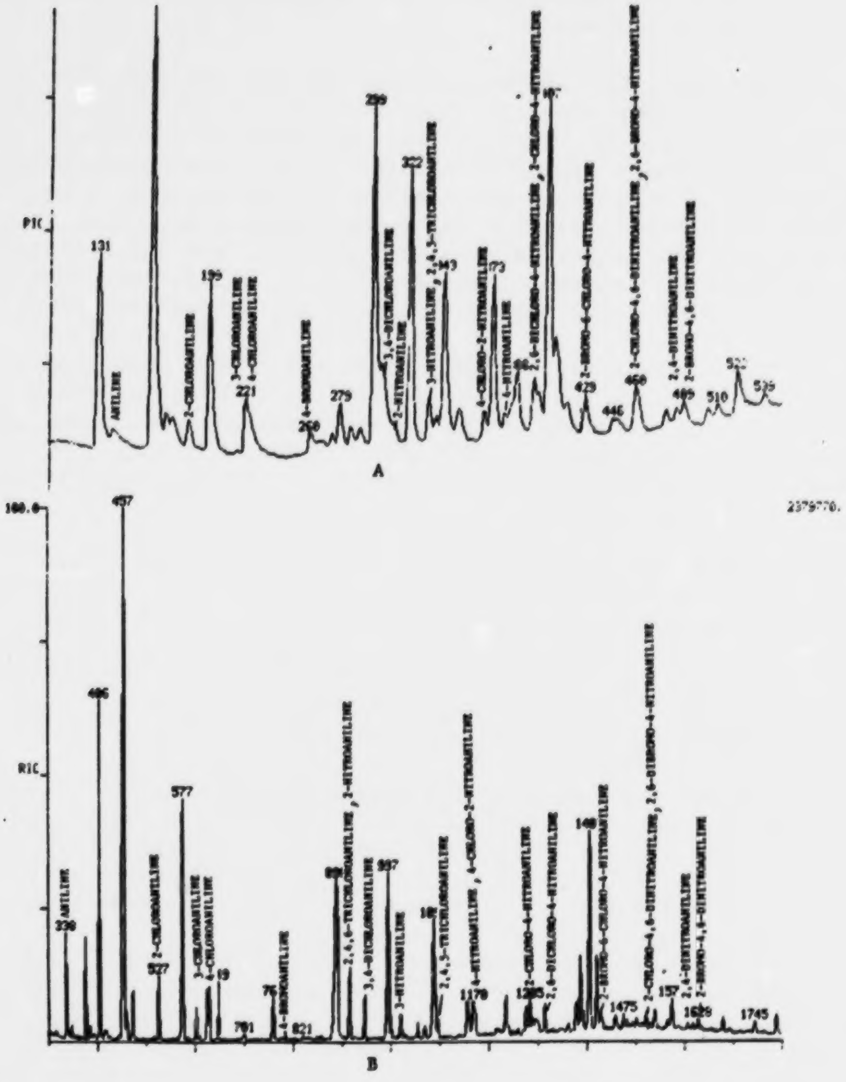

Figure 11. Reconstructed ion chromatography of 25 µg/L spike of Wastewater 3a on SP-2250 packed column (A) and SE-54 FSCC (B).

The Sludge Method (Appendix E) involves homogenization/extraction of the sludge with methylene chloride at pH 11, cleanup of the methylene chloride extract using Florisil or gel permeation chromatography (GPC) and quantification of the compounds on packed or capillary column GC-MS as described in U.S. EPA Method 625. Only capillary column GC-MS was employed in this portion of the study.

Initially, GPC characteristics for the anilines were evaluated. The GPC retention time data are shown in Table 44 and a corresponding GPC calibration run is shown in Figure 12. As shown by these data, all of the anilines elute between 32 and 38 minutes, with trichloroaniline eluting last, presumably due to a reversed-phase effect. Consequently, a collection range from 28-40 minutes was selected for method evaluation. This represents collection from the valley between dioctyl phthalate and 2,4-dinitro-6-methylphenol to the valley between pentachlorophenol and sulfur. This range excludes long chain hydrocarbons, fatty acids, and triglycerides.

The Florisil elution data using the Sludge Method have been presented earlier (Table 19) and the compounds were found to elute primarily in the fourth (methylene chloride) and fifth (5% acetone in methylene chloride) fractions. One compound, 2,4,6-trichloroaniline was eluted in the third fraction (50% methylene chloride in hexane). Consequently, these three fractions were combined prior to concentration and analysis.

POTW sludge sample (Columbus) was spiked with 1 mg/L of each aniline, and extracted according to the Sludge Method. The extract was dried, concentrated, split in half for parallel GPC and Florisil cleanup, and analyzed by capillary column GC-MS. Equivalent recoveries (within 20%) were obtained using the two methods, except that 2,4,6-trichloroaniline recovery was only 17% by GPC compared to 50% by Florisil cleanup. Recoveries for the various compounds were in the range of 50-100%.

The GPC treated extract was colorless whereas the Florisil treated extract was dark brown. However, for GPC the GC-MS chromatogram was more complex, and had a large unresolved "hump", as illustrated for an unspiked extract in Figure 13 (using GC-FID). Both extracts contained late eluting peaks which required the use of a one hour sample analysis time.

On the basis of these data, the Florisil cleanup option was selected for use in the method validation efforts, since a less complex chromatogram was obtained compared to GPC cleanup. The Sludge Method was validated by acquiring MDL, analytical curve, and high level spike data for relevant samples using the Florisil cleanup and capillary column GC-MS options. The only modification made was the use of ethylene dichloride as the injection solvent in order to improve capillary column performance for splitless injections.

MDL data for Columbus and Cincinnati POTW sludges (both approximately 5% solids) are shown in Tables 45 and 46. The MDLs, calculated as described in Appendix B, were similar for the two samples, ranging from 10-350 µg/L. Significant levels of aniline and chlorinated anilines were observed in the unspiked samples. Percent recoveries were generally greater

TABLE 44. GPC DATA ON ANILINE COMPOUNDS

Compound	Elution Time[a] (minutes)
Aniline	35
4-Nitroaniline	34
3-Nitroaniline	34
2-Nitroaniline	34.5
2,4-Dinitroaniline	32.5
4-Chloroaniline	36
3-Chloroaniline	36.5
2-Chloroaniline	36
2-Chloro-4-nitroaniline	34
4-Bromoaniline	36.5
2-Chloro-4,6-dinitroaniline	33
2-Bromo-4,6-dinitroaniline	32
4-Chloro-2-nitroaniline	36
3,4-Dichloroaniline	36.5
2,6-Dichloro-4-nitroaniline	35
2-Bromo-6-chloro-4-nitroaniline	33.5
2,6-Dibromo-4-nitroaniline	34
2,4,5-Trichloroaniline	38

(a) Using a 25mmID x 600mm glass column packed with 70g of Bio-Beads S-X3 and elution with methylene chloride at 5 ml/min.

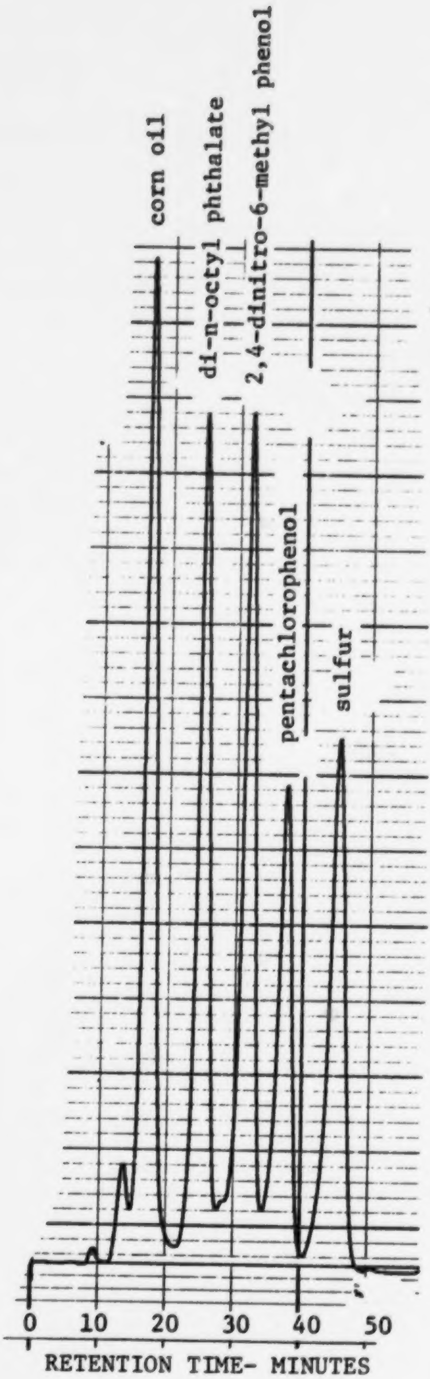

Figure 12. Chromatogram of GPC calibration standard using Bio-Beads S-X3 and elution with methylene chloride.

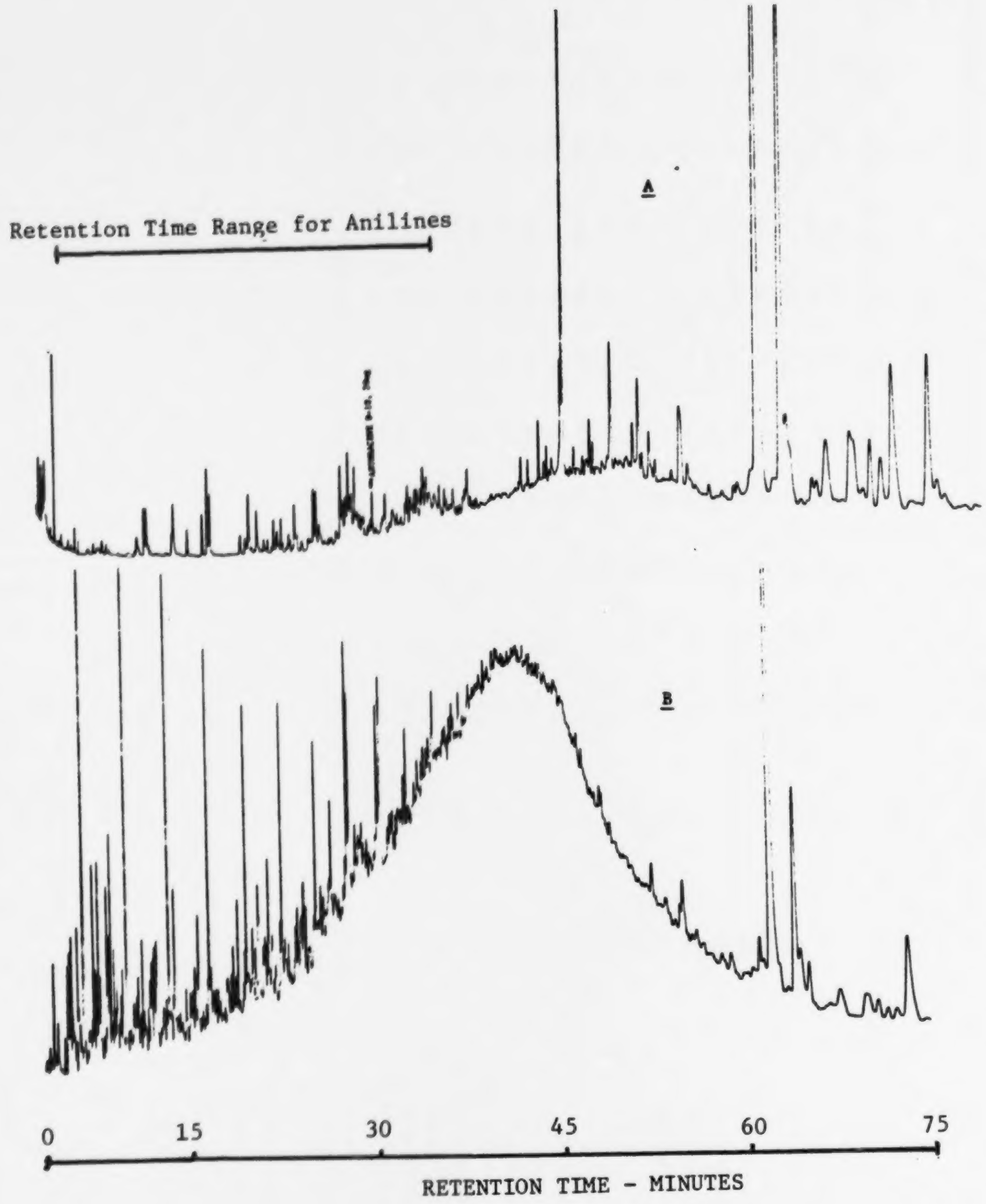

Figure 13. GC-FID chromatograms of an unspiked Columbus POTW sludge extract fractionated using a Florisil column (A), and GPC (B).

TABLE 45. RESULTS OF METHOD DETECTION LIMIT (MDL) DETERMINATION FOR ANILINES IN COLUMBUS POTW SLUDGE

			Amount Found in Given Spiked Replicate [b], µg/L										
Compound	Amount Spiked, µg/L	Amount Found in Unspiked Sample, µg/L [a]	#1	#2	#3	#4	#5	#6	#7	Average	SD [c]	MDL [d]	Average Percent Recovery
Aniline	250	2.0	50	74	64	41	81	86	78	68	17	53	26
2-Chloroaniline	100	10	73	70	69	73	65	69	66	69	3.1	9.7	59
3-Chloroaniline	100	7.7	84	54	78	93	100	134	100	92	24	75	84
4-Chloroaniline [e]	100	7.7	84	54	78	93	100	134	100	92	24	75	84
4-Bromoaniline [e]	100	ND [f]	25	35	28	36	49	53	48	39	11	35	39
3,4-Dichloroaniline	100	32	150	170	150	200	150	210	160	170	25	79	140
2,4,6-Trichloroaniline	100	ND	70	75	70	80	73	74	69	73	3.8	12	73
2,4,5-Trichloroaniline	100	ND	85	79	70	81	75	73	69	76	5.9	19	76
2-Nitroaniline	100	ND	76	96	89	90	74	81	78	83	8.3	26	83
3-Nitroaniline	250	ND	130	240	150	210	150	160	160	170	39	123	68
4-Nitroaniline	500	ND	340	430	300	350	400	430	320	370	53	170	74
2,4-Dinitroaniline	500	ND	130	160	120	120	130	110	94	120	20	63	24
4-Chloro-2-nitroaniline	250	ND	160	210	180	190	180	180	150	180	20	63	72
2-Chloro-4-nitroaniline	250	ND	220	230	210	180	170	230	180	200	26	82	80
2,6-Dichloro-4-nitroaniline	250	ND	150	190	190	190	210	200	160	180	21	66	72
2,6-Dibromo-4-nitroaniline	250	ND	100	130	94	81	110	130	93	110	19	60	44
2-Bromo-6-chloro-4-nitroaniline	250	ND	140	190	160	170	220	170	160	170	26	82	68
2-Chloro-4,6-dinitroaniline	500	ND	52	110	73	99	150	230	170	130	61	190	26
2-Bromo-4,6-dinitroaniline	500	ND	140	270	40	65	71	100	100	110	76	240	22

(a) Average of five replicate analyses.

(b) An 80-ml sample of sludge was used.

(c) SD= standard deviation.

(d) Method detection limit as determined using EPA protocol.

(e) 4-Chloroaniline and 3-chloroaniline are not chromatographically resolved. The values shown are based on one-half of the total response for the two isomers.

(f) ND = not detected.

TABLE 46. RESULTS OF METHOD DETECTION LIMIT (MDL) DETERMINATION FOR ANILINES IN CINCINNATI POTW SLUDGE

Compound	Amount Spiked, µg/L	Amount Found in Unspiked Sample, µg/L[a]	Amount Found in Given Spiked Replicate[b], µg/L										
			#1	#2	#3	#4	#5	#6	#7	Average	SD[c]	MDL[d]	Average Percent Recovery
Aniline	250	18	110	70	50	80	37	43	46	62	26	82	18
2-Chloroaniline	100	28	95	69	55	69	55	54	56	65	15	47	37
3-Chloroaniline[e]	100	9.5	52	38	28	40	31	31	33	36	8.2	26	27
4-Chloroaniline[e]	100	9.5	52	38	28	40	31	31	33	36	8.2	26	27
4-Bromoaniline	100	ND[f]	40	28	24	28	25	25	27	28	5.5	17	28
3,4-Dichloroaniline	100	51	100	75	74	90	89	92	84	86	9.4	30	35
2,4,6-Trichloroaniline	100	30	74	55	57	70	46	50	50	57	11	35	27
2,4,5-Trichloroaniline	100	ND	55	41	43	58	51	53	53	51	6.3	20	51
2-Nitroaniline	100	ND	78	52	44	54	33	36	47	49	15	47	49
3-Nitroaniline	250	ND	120	71	49	85	62	70	77	76	22	69	30
4-Nitroaniline	500	ND	250	210	65	210	160	180	180	180	58	180	36
2,4-Dinitroaniline	500	ND	160	120	36	250	250	200	170	170	76	240	34
4-Chloro-2-nitroaniline	250	ND	150	110	89	130	83	100	110	110	23	72	44
2-Chloro-4-nitroaniline	250	ND	170	140	110	150	97	110	130	130	26	82	52
2,6-Dichloro-4-nitroaniline	250	ND	150	110	130	170	110	140	140	140	22	69	56
2,6-Dibromo-4-nitroaniline	250	ND	100	94	80	170	130	130	130	120	30	94	48
2-Bromo-6-chloro-4-nitroaniline	250	ND	140	130	110	200	140	160	160	150	29	91	60
2-Chloro-4,6-dinitroaniline	500	ND	110	54	25	280	200	200	190	150	91	290	30
2-Bromo-4,6-dinitroaniline	500	ND	120	79	30	300	290	240	230	180	110	350	36

(a) Average of five replicate analyses.

(b) An 80-ml sample of sludge was used.

(c) SD = standard deviation.

(d) Method detection limit as determined using EPA protocol.

(e) 4-Chloroaniline and 3-chloroaniline are not chromatographically resolved. The values shown are based on one-half of the total response for the two isomers.

(f) ND = not detected.

than 50% for the Columbus sample but were only 30-50% for most compounds in the Cincinnati sample. The higher amount of industrial influent represented by the Cincinnati POTW may have been a contributing factor in the lower recoveries obtained for this sample.

High spike level data for the Columbus POTW sample are shown in Table 47. Generally, recoveries were 100-130%, although aniline had only 64% recovery. An extremely large concentration of 3,4-dichloroaniline was observed in the spiked samples. Subsequently, an additional unspiked aliquot was analyzed and found to contain a much larger quantity of 3,4-dichloroaniline (3400 µg/L) than had been previously obtained. Since it did not seem reasonable that the sample could have become contaminated with only 3,4-dichlotoaniline, an alternate explanation for this result seemed more reasonable. The sludge sample had been stored at 4°C for approximately two weeks and therefore some sample components may have been degraded to form 3,4-dichloroaniline.

Collection of a fresh sample and reanalysis after several weeks storage confirmed that the 3,4-dichloroaniline level increased dramatically. A similar result was obtained for Cincinnati POTW sludge. A common ingredient in deodorant soaps, trichlorocarban (N-(4-chlorophenyl)-N'-(3,4-dichlorophenyl)urea) contains both 3,4-dichloroaniline and 4-chloroaniline moieties. Possibly biological or chemical degradation of trichlorocarban to these species in the sludge matrix is a reasonable explanation for these observations. However, confirmation of this hypothesis was considered beyond the scope of this study.

Analytical curve data for the aniline in Columbus POTW sludge are presented in Table 48. Since these samples were processed at the same time as the high spike level samples, the problem with high 3,4-dichloroaniline background is evident. In addition, the data for the stored unspiked sample indicated an elevated level of 3- or 4-chloroaniline (480 µg/L) compared to the original value of 15 µg/L. This observation further substantiates the hypothesis that trichlorocarban degradation may be responsible for the elevated levels in the stored samples. The reason for the high (>100%) recoveries in high spike Columbus POTW sample (Table 47) is not clear, although an instrument calibration error is one possible explanation.

The analytical curve data indicate that quantitative recovery is obtained for most of the compounds at the medium and high spike levels, with poorer recoveries obtained for the lowest spike level. Aniline recovery was only 67% at the high spike level, perhaps because of its greater water solubility. Linear least square analysis results of the analytical curve data are presented in Table 49.

Results for the 1-hour and 24-hour liquid/solid equilibration experiments are presented in Tables 50 and 51. The data are summarized in Table 52. The relevant conclusions drawn from the data shown in Tables 50-52 are summarized as follows:

TABLE 47. RESULTS FOR THE DETERMINATION OF ANILINES SPIKED AT A HIGH LEVEL IN COLUMBUS POTW SLUDGE

Compound	Amount Spiked, μg/L	Amount Found in Given Spiked Replicate [a], μg/L									
		#1	#2	#3	#4	#5	#6	#7	Average	SD [b]	Average Percent Recovery
Aniline	2500	1400	1600	1800	1800	1700	1300	1400	1600	190	64
2-Chloroaniline	1000	1000	1000	1200	1200	1000	1100	900	1100	110	110
3-Chloroaniline [c]	1000	1200	1300	1400	1400	1300	1300	1100	1300	110	130
4-Chloroaniline [c]	1000	1200	1300	1400	1400	1300	1300	1100	1300	110	130
4-Bromoaniline	1000	900	900	1100	1100	1000	1000	900	990	90	99
3,4-Dichloroaniline	1000	5900	6400	7900	6500	5900	6800	5400	6400	810	300 [d]
2,4,6-Trichloroaniline	1000	1000	1000	1200	1200	1100	1300	1000	1100	120	110
2,4,5-Trichloroaniline	1000	1100	1100	1400	1200	1100	1400	1100	1200	140	120
2-Nitroaniline	1000	1200	1300	1500	1500	1300	1500	1200	1400	140	140
3-Nitroaniline	2500	3000	3000	3500	3600	3200	3500	2800	3200	310	130
4-Nitroaniline	5000	6500	6000	7400	7200	6400	6900	5600	6600	640	130
2,4-Dinitroaniline	5000	5400	6800	7700	6900	4300	6000	5000	6000	1200	120
4-Chloro-2-nitroaniline	2500	2900	2900	3500	3500	2600	3300	2700	3100	370	120
2-Chloro-4-nitroaniline	2500	3500	3500	4200	4000	3200	3900	3100	3600	420	140
2,6-Dichloro-4-nitroaniline	2500	3200	3200	3900	3800	2700	3600	2900	3300	450	130
2,6-Dibromo-4-nitroaniline	2500	3300	3500	4300	3900	2300	3200	2600	3300	700	130
2-Bromo-6-chloro-4-nitroaniline	2500	3700	3700	4600	4200	2900	3700	3100	3700	590	150
2-Chloro-4,6-dinitroaniline	5000	5800	6600	8000	7300	4500	6200	4800	6200	1300	120
2-Bromo-4,6-dinitroaniline	5000	5600	6600	8200	6900	3900	6300	4600	6000	1500	120

(a) An 80-ml sample of sludge was used. Background levels are given in Table 45.

(b) SD = standard deviation.

(c) 4-Chloroaniline and 3-chloroaniline are not chromatographically resolved. The values shown are based on one-half of the total response for the two isomers.

(d) The 3,4-dichloroaniline background level was shown to increase dramatically upon storage. Therefore the background level used for determining percent recovery is that found for the stored sample (3400 μg/L). This value is subject to error because of the high background level relative to the spike level.

TABLE 48. RESULTS OF ANALYTICAL CURVE DETERMINATION FOR ANILINES IN COLUMBUS POTW SLUDGE

Compound	Amount Spiked, µg/L	Amount Found in Given Spiked Sample, µg/L			Percent Recovery [a]
		Rep 1	Rep 2	Average	
Aniline	250	b	b	68	27
Aniline	750	450	410	430	57
Aniline	2500	c	c	1600	64
Aniline	5000	3200	3500	3400	67
2-Chloroaniline	100	b	b	69	63
2-Chloroaniline	300	250	260	260	85
2-Chloroaniline	1000	c	c	1100	110
2-Chloroaniline	2000	1700	2000	1900	93
3-Chloroaniline+4-Chloroaniline	200	b	b	180	90 [d]
3-Chloroaniline+4-Chloroaniline	600	980	1000	990	85 [d]
3-Chloroaniline+4-Chloroaniline	2000	c	c	2600	110 [d]
3-Chloroaniline+4-Chloroaniline	4000	3600	4200	3900	86 [d]
4-Bromoaniline	100	b	b	39	39
4-Bromoaniline	300	230	240	240	80
4-Bromoaniline	1000	c	c	990	99
4-Bromoaniline	2000	1700	2100	1900	95
3,4-Dichloroaniline	100	b	b	170	130 [e]
3,4-Dichloroaniline	300	4300	4600	4500	370 [e]
3,4-Dichloroaniline	1000	c	c	6400	300 [e]
3,4-Dichloroaniline	2000	6000	6800	6400	150 [e]
2,4,6-Trichloroaniline	100	b	b	73	73
2,4,6-Trichloroaniline	300	69	280	170	57
2,4,6-Trichloroaniline	1000	c	c	1100	110
2,4,6-Trichloroaniline	2000	1500	2200	1900	93
2,4,5-Trichloroaniline	100	b	b	76	76
2,4,5-Trichloroaniline	300	140	310	230	77
2,4,5-Trichloroaniline	1000	c	c	1200	120
2,4,5-Trichloroaniline	2000	1700	2400	2100	110

TABLE 48. (Continued)

Compound	Amount Spiked, μg/L	Amount Found in Given Spiked Sample, μg/L			Percent Recovery[a]
		Rep 1	Rep 2	Average	
2-Nitroaniline	100	b	b	83	83
2-Nitroaniline	300	310	340	330	110
2-Nitroaniline	1000	c	c	1400	140
2-Nitroaniline	2000	2100	2700	2400	120
3-Nitroaniline	250	b	b	170	52
3-Nitroaniline	750	730	800	770	110
3-Nitroaniline	2500	c	c	3200	130
3-Nitroaniline	5000	11000	6100	8600	170
4-Nitroaniline	500	b	b	370	74
4-Nitroaniline	1500	1500	1800	1700	110
4-Nitroaniline	5000	c	c	6600	130
4-Nitroaniline	10000	5400	14000	7300	73
2,4-Dinitroaniline	500	b	b	120	24
2,4-Dinitroaniline	1500	1500	2100	1800	120
2,4-Dinitroaniline	5000	c	c	6000	120
2,4-Dinitroaniline	10000	12000	8500	10000	100
4-Chloro-2-nitroaniline	250	b	b	180	72
4-Chloro-2-nitroaniline	750	740	940	840	110
4-Chloro-2-nitroaniline	2500	c	c	3100	120
4-Chloro-2-nitroaniline	5000	4600	5900	5200	100
2-Chloro-4-nitroaniline	250	b	b	200	80
2-Chloro-4-nitroaniline	750	790	1100	950	130
2-Chloro-4-nitroaniline	2500	c	c	3600	140
2-Chloro-4-nitroaniline	5000	5600	6900	6300	130
2,6-Dichloro-4-nitroaniline	250	b	b	180	72
2,6-Dichloro-4-nitroaniline	750	790	930	860	110
2,6-Dichloro-4-nitroaniline	2500	c	c	3300	130
2,6-Dichloro-4-nitroaniline	5000	4900	6000	5500	110

TABLE 48. (Continued)

Compound	Amount Spiked, µg/L	Amount Found in Given Spiked Sample, µg/L			Percent Recovery[a]
		Rep 1	Rep 2	Average	
2,6-Dibromo-4-nitroaniline	250	b	b	110	44
2,6-Dibromo-4-nitroaniline	750	810	1100	960	130
2,6-Dibromo-4-nitroaniline	2500	c	c	3300	130
2,6-Dibromo-4-nitroaniline	5000	4200	5100	4700	94
2-Bromo-6-chloro-4-nitroaniline	250	b	b	170	68
2-Bromo-6-chloro-4-nitroaniline	750	900	1100	1000	130
2-Bromo-6-chloro-4-nitroaniline	2500	c	c	3700	150
2-Bromo-6-chloro-4-nitroaniline	5000	4700	6200	5500	110
2-Chloro-4,6-dinitroaniline	500	b	b	130	26
2-Chloro-4,6-dinitroaniline	1500	1300	1500	1400	93
2-Chloro-4,6-dinitroaniline	5000	c	c	6200	120
2-Chloro-6,6-dinitroaniline	10000	10000	9100	9600	96
2-Bromo-4,6-dinitroaniline	500	b	b	110	22
2-Bromo-4,6-dinitroaniline	1500	1400	1800	1600	110
2-Bromo-4,6-dinitroaniline	5000	c	c	6000	120
2-Bromo-4,6-dinitroaniline	10000	12000	8400	10000	100

(a) Corrected for background levels given in Table 45.
(b) Seven replicates analyzed. See Table 45 for individual values.
(c) Seven replicates analyzed. See Table 47 for individual values.
(d) Percent recoveries corrected for background on all samples except 200 µg/L spikes. Background level used was 480 µg/L.
(e) Background level of 3400 µg/L was used for this calculation. Low spike level indicates negative recovery and is not meaningful due to the high background level.

TABLE 49. LINEAR LEAST SQUARES PARAMETERS FOR ANALYTICAL CURVE DATA FOR ANILINES IN POTW SLUDGE (COLUMBUS)

Compound	Y-Intercept [a]	Slope	Correlation Coefficient
Aniline	-51	0.68	0.999
2-Chloroaniline	14	0.97	0.996
3-Chloroaniline-4-Chloroaniline	125	0.85	0.992
4-Bromoaniline	-28	0.97	0.999
3,4-Dichloroaniline	215	1.70	0.882
2,4,6-Trichloroaniline	-17	0.98	0.994
2,4,5-Trichloroaniline	0.63	1.10	0.995
2-Nitroaniline	11	1.20	0.996
3-Nitroaniline	-380	1.70	0.992
4-Nitroaniline	-76	1.30	0.996 [b]
2,4-Dinitroaniline	270	1.01	0.992
4-Chloro-2-nitroaniline	89	1.10	0.995
2-Chloro-4-nitroaniline	54	1.30	0.997
2,6-Dichloro-4-nitroaniline	80	1.10	0.994
2,6-Dibromo-4-nitroaniline	230	0.96	0.979
2-Bromo-6-chloro-4-nitroaniline	200	1.10	0.985
2-Chloro-4,6-dinitroaniline	61	1.00	0.988
2-Bromo-4,6-dinitroaniline	150	1.00	0.993

(a) x-axis = expected concentration (µg/L)
y-axis = found concentration (µg/L)

(b) Calculated without 10,000 µg/L data point.

TABLE 50. RESULTS FOR THE EQUILIBRATION OF ANILINES WITH COLUMBUS POTW SLUDGE FOR ONE HOUR TIME PERIOD[a]

Compound	Amount Spiked (µg)	Amount Found in Aqueous Phase (µg)				Amount Found in Solid Phase (µg)				Total Recovery		Partition Ratio[b]
		Replicate 1	Replicate 2	Replicate 3	Average	Replicate 1	Replicate 2	Replicate 3	Average	µg	%	
Aniline	400	66	82	90	79	38	27	29	31	110	28	.92
2-Chloroaniline	160	33	41	43	39	62	57	57	59	98	61	3.5
3-Chloroaniline[c]	160	15	17	17	16	71	52	58	60	76	48	8.7
4-Chloroaniline[c]	160	15	17	17	16	71	52	58	60	76	48	8.7
4-Bromoaniline	160	28	31	33	31	58	49	54	54	85	53	4.1
3,4-Dichloroaniline	160	15	17	18	17	120	99	110	110	130	81	15
2,4,6-Trichloroaniline	160	5.6	7.0	6.7	6.4	76	92	87	85	90	56	31
2,4,5-Trichloroaniline	160	6.1	6.6	6.3	6.3	130	110	120	120	130	81	44
2-Nitroaniline	160	42	43	47	44	75	63	68	69	110	69	3.7
3-Nitroaniline	400	110	110	120	110	95	84	100	93	200	50	2.0
4-Nitroaniline	800	270	280	330	290	260	260	290	270	560	70	2.2
2,4-Dinitroaniline	800	200	160	190	180	420	390	290	370	550	69	4.8
4-Chloro-2-nitroaniline	400	36	35	38	36	250	210	240	230	270	68	15
2-Chloro-4-nitroaniline	400	59	57	65	60	240	220	240	230	290	73	8.9
2,6-Dichloro-4-nitroaniline	400	24	24	25	24	280	250	280	270	290	73	26
2,6-Dibromo-4-nitroaniline	400	14	13	12	13	400	260	270	310	320	80	56
2-Bromo-6-chloro-4-nitroaniline	400	17	16	17	17	340	290	330	320	340	85	44
2-Chloro-4,6-dinitroaniline	800	37	32	49	39	30	90	110	77	120	15	4.6
2-Bromo-4,6-dinitroaniline	800	36	26	38	33	88	100	120	100	130	16	7.1

(a) An 80-ml aliquot of municipal sludge containing approximately 4% solids was equilibrated with the aniline compounds. The sludge was then centrifuged to remove a major portion of the aqueous phase, approximately 70 mL. The remaining 30 mL is called the "solid" phase.

(b) Partition ratio = $\frac{\mu g \text{ in solid phase}/30 \text{ mL}}{\mu g \text{ in water phase}/70 \text{ mL}}$

(c) 4-Chloroaniline and 3-chloroaniline are not chromatographically resolved. The values shown are based on one-half of the total response for the two isomers.

TABLE 51. RESULTS FOR THE EQUILIBRATION OF ANILINES WITH COLUMBUS POTW SLUDGE FOR 24 HOURS[a]

Compound	Amount Spiked (μg)	Amount Found in Aqueous Phase (μg) Replicate 1	Replicate 2	Average	Amount Found in Solid Phase (μg) Replicate 1 [c]	Total Recovery μg	%	Partition Ratio [b]
Aniline	400	96	100	98	37	140	34	.86
2-Chloroaniline	160	48	47	48	60	110	69	2.9
3-Chloroaniline [d]	160	17	19	18	61	79	49	7.9
4-Chloroaniline [d]	160	17	19	18	61	79	49	7.9
4-Bromoaniline	160	29	33	31	55	86	54	4.1
3,4-Dichloroaniline	160	16	18	17	110	130	81	15
2,4,6-Trichloroaniline	160	9.0	8.7	8.9	74	83	52	19
2,4,5-Trichloroaniline	160	8.2	8.4	8.3	120	130	81	34
2-Nitroaniline	160	46	52	49	71	120	75	3.4
3-Nitroaniline	400	95	120	110	72	180	45	1.5
4-Nitroaniline	800	240	360	300	300	600	75	2.3
2,4-Dinitroaniline	800	33	87	60	220	280	35	8.6
4-Chloro-2-nitroaniline	400	31	39	35	200	240	60	13
2-Chloro-4-nitroaniline	400	45	65	55	220	280	70	9.3
2,6-Dichloro-4-nitroaniline	400	22	26	24	230	250	63	22
2,6-Dibromo-4-nitroaniline	400	13	12	13	210	220	55	38
2-Bromo-6-chloro-4-nitroaniline	400	15	19	17	270	290	73	37
2-Chloro-4,6-dinitroaniline	800	1.7	2.1	1.9	50	52	6.5	61
2-Bromo-4,6-dinitroaniline	800	1.5	ND [e]	1.5	64	66	8.3	100

(a) An 80-mL aliquot of municipal sludge containing approximately 4% solids was equilibrated with the aniline compounds. The sludge was then centrifuged to remove a major portion of the aqueous phase, approximately 70 mL. The remaining 30 mL is called the "solid" phase.

(b) Partition ratio = $\frac{\mu g \text{ in solid phase}/30 \text{ mL}}{\mu g \text{ in aqueous phase}/70 \text{ mL}}$

(c) Only one sample analyzed. Second sample was lost during processing.

(d) 4-Chloroaniline and 3-chloroaniline are not chromatographically resolved. The values shown are based on one-half of the total response for the two isomers.

(e) ND = Not detected. Not included in calculating average value.

TABLE 52. SUMMARY OF RESULTS FOR THE EQUILIBRATION OF ANILINES WITH COLUMBUS POTW SLUDGE[a]

	Spike	% Recovery		Partition Ratio [b]	
Compound	Level (µg)	1 hr	24 hr	1 hr	24 hr
Aniline	400	28	34	.92	.88
2-Chloroaniline	160	61	69	3.5	2.9
3-Chloroaniline [c]	160	48	49	8.7	7.9
4-Chloroaniline [c]	160	48	49	8.7	7.9
4-Bromoaniline	160	53	54	4.1	4.1
3,4-Dichloroaniline	160	81	81	15	15
2,4,6-Trichloroaniline	160	56	52	31	19
2,4,5-Trichloroaniline	160	81	81	44	34
2-Nitroaniline	160	69	75	3.7	3.4
3-Nitroaniline	400	50	45	2.0	1.5
4-Nitroaniline	800	70	75	2.2	2.3
2,4-Dinitroaniline	800	69	35	4.8	8.6
4-Chloro-2-nitroaniline	400	68	60	15	13
2-Chloro-4-nitroaniline	400	73	70	8.9	9.3
2,6-Dichloro-4-nitroaniline	400	73	63	26	22
2,6-Dibromo-4-nitroaniline	400	80	55	56	38
2-Bromo-6-chloro-4-nitroaniline	400	85	73	44	37
2-Chloro-4,6-dinitroaniline	800	15	6.3	4.6	61
2-Bromo-4,6-dinitroaniline	800	16	8.3	7.1	100

(a) An 80-ml aliquot of municipal sludge containing approximately 4% solids was equilibrated with the aniline compounds. The sludge was then centrifuged to remove a major portion of the aqueous phase, approximately 70 mL. The remaining 30 ml is called the "solid" phase.

(b) Partition ratio $= \frac{\mu g \text{ in solid phase}/30 \text{ mL}}{\mu g \text{ in water phase}/70 \text{ mL}}$

(c) 4-Chloroaniline and 3-chloroaniline are not chromatographically resolved. The values shown are based on one-half of the total response for the two isomers.

- Recoveries were generally 50-80% although aniline, 2-chloro-4,6-dinitroaniline, and 2-bromo-4,6-dinitroaniline gave lower recoveries.

- Recoveries using 1-hour and 24-hour equilibration times for most compounds were essentially identical, indicating that the compounds are stable in the matrix under the experimental conditions employed. However, 2,4-dinitroaniline, 2-chloro-4,6-dinitroaniline, and 2-bromo-4,6-dinitroaniline recoveries were lower using a 24-hour equilibration period.

- The partition ratios (solid/liquid) for all the anilines are much greater than 1, with the exception of aniline which was ∿ 1. The three mononitroanilines also had relatively low partition ratios, presumably because of their greater water solubility.

- The partition ratio for most compounds was unaffected by equilibration time, the only exception being 2-chloro-4,6-dinitroaniline and 2-bromo-4,6-dinitroaniline. Perhaps the presence of two bulky groups adjacent to the amine group is responsible for the slowly equilibration ratio observed for these compounds. This result indicates that the liquid/solid equilibrium is rapidly established after spiking.

Although these data are not directly required for the validation of an analytical method for sludge, they are useful in understanding the behavior of anilines in sludge, and may be useful for assessing wastewater treatment methods.

SECTION 7

REFERENCES

1. Interagency Testing Committee; Receipt of Fourth Report and Request for Comments. Federal Register, Vol. 44, No. 107, pp. 31866-31889, Friday, June 1, 1979.

2. Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater. EPA-600/4-82-057, U.S. Environmental Protection Agency, Cincinnati, OH, 1982. Method 625-Base/Neutrals and Acids.

3. Development of Analytical Test Procedures for the Measurement of Organic Priority Pollutants in Sludge. U.S. Environmental Protection Agency, Cincinnati, OH, Final Report on Contract No. 68-03-2695 (in preparation).

4. Venturelle, V.S., V.M. Gualario, and R.E. Lang. A Rapid Gas-Liquid Chromatographic Method for the Determination of Halogenonitroanilines and Aminoanisoles. J. Chrom. Sci., 11:379-383, 1973.

5. Farroha, S.M. and S.S. Emeish. Gas Chromatographic Separation of Aromatic Amines, Nitriles, and Hydrocarbons-I. Talanta, 22:121-128, 1975.

6. Willeboordse, F., Q. Quick, and E.T. Bishop. Direct Gas Chromatographic Analysis of Isomeric Diaminotoluenes. Anal. Chem., 40(10):1455-1458, 1968.

7. Bowen, B.E. Determination of Aromatic Amines by an Adsorption Technique with Flame Ionization Gas Chromatography. Anal. Chem., 48(11):1584-1587, 1976.

8. Krasuska, E. and W. Celler. Gas Chromatographic Separation of Chloronitrobenzenes, Nitroanilines, and Phenylenediamines. J. Chromatog., 147:470-472, 1978.

9. Zielinski, W.L., L. Fishbein, and R.O. Thomas. Relationship of Structure to Sensitivity in Electron Capture Analysis:III. Chloronitrobenzenes, Anilines, and Related Derivatives. J. Chromatog., 30:77-85, 1967.

10. Reference 2. Method 607-Nitrosamines.

11. Bradway, D.E. and T. Shafik. Electron Capture Gas Chromatographic Analysis of the Amine Metabolites of Pesticides: Derivatization of Anilines. J. Chrom. Sci., 15:322-328, 1977.

12. Lopez-Avila, V. and R. Northcutt. Application of the Hall Electrolytic Conductivity Detector for the Analysis of Chloronitroanilines in POTW Sludges. Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Abstract No. 224, 1981.

13. Lores, E.M., D.W. Bristol, and R.F. Moseman. Determination of Halogenated Anilines and Related Compounds by HPLC with Electrochemical and UV Detection. J. Chrom. Sci., 16:358-362, 1978.

14. Lores, E.M., F.C. Meekins, and R.F. Moseman. Determination of Halogenated Anilines in Urine by High Performance Liquid Chromatography with an Electrochemical Detector. J. Chromatog., 188:412-416, 1980.

15. Glaser, J.A., D.L. Foerst, G.D. McKee, S.A. Quave, and W.L. Budde, Trace Analyses for Wastewaters. Env. Sci. Tech., 15(12):1426-1435, 1981.

16. Riggin, R.M. and C.C. Howard. Determination of Benzidines in Industrial and Municipal Wastewaters. EPA-600/54-82-022, U.S. Environmental Protection Agency, Cincinnati, OH, 1982.

APPENDIX A

ANILINE AND SELECTED SUBSTITUTED DERIVATIVES

U.S. EPA METHOD 650

1. Scope and Application

1.1 This method covers the determination of aniline and certain derivatives of aniline. The following parameters can be determined by this method:

Parameter	CAS No.
Aniline	62-53-3
2-Chloroaniline	95-51-2
3-Chloroaniline	108-42-9
4-Chloroaniline	106-47-8
4-Bromoaniline	106-40-1
3,4-Dichloroaniline	95-76-1
2,4,6-Trichloroaniline	634-93-5
2,4,5-Trichloroaniline	636-30-6
2-Nitroaniline	88-74-4
3-Nitroaniline	99-09-2
4-Nitroaniline	100-01-6
2,4-Dinitroaniline	97-02-9
4-Chloro-2-nitroaniline	89-63-4
2-Chloro-2-nitroaniline	121-87-9
2,6-Dichloro-4-nitroaniline	99-30-9
2,6-Dibromo-4-nitroaniline	827-94-1
2-Bromo-6-chloro-4-nitroaniline	99-29-6
2-Chloro-4,6-dinitroaniline	3531-19-9
2-Bromo-4,6-dinitroaniline	1817-73-8

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. U.S. EPA Method 625, with minor modifications (1) provides gas chromatograph/mass spectrometer (GC-MS) conditions appropriate for the qualitative and quantitative confirmation of results for the parameters listed above using the extract produced by this method.

1.3 This method provides for fused silica capillary column (FSCC) and glass packed column approaches to the determination of the aniline compounds. The SE-54 FSCC procedure does not adequately resolve the following two pairs of compounds: 2-nitroaniline and 2,4,6-trichloroaniline; and 4-nitroaniline and 4-chloro-2-nitroaniline. Only partial resolution of 3-chloroaniline and 4-chloroaniline is achieved. Unless the purpose for the analysis can be served by reporting the sum of an unresolved pair, the packed column or alternate capillary column gas chromatographic approach must be used for these compounds. The alternate fused silica capillary column (SE-30) gives resolution of these compound pairs but fails to resolve 2,6-dibromo-4-nitroaniline and 2,4-dinitroaniline and gives only partial resolution of 3-chloroaniline and 4-chloroaniline. The packed column gas chromatographic approach will give a partial separation for all the aniline compounds. However, resolution of anilines from other matrix components will generally be better when the capillary column is employed. Guidelines for selecting alternate capillary columns are given in Section 12.1.

1.4 The method detection limit (MDL, defined in Section 15) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.5 Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternate test procedures under applicable regulations.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately 1-liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to cyclohexane, then to toluene during concentration to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by GC with nitrogen phosphorus detection (GC-NPD) (1).

2.2 This method provides Florisil column cleanup procedures to aid in the elimination of interferences which may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned (2). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap and distilled water. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials such as PCBs may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedures in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified (3-5) for the information of the analyst.

4.2 Aniline, 4-chloroaniline, and 2,4,6-trichloroaniline have been found to induce tumors in laboratory animals (6). Primary standards of these toxic chemicals should be prepared in a hood.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber glass, 1-liter or 1-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel - 2000-mL with Teflon stopcock.

5.2.2 Drying column - Chromatographic column 400 mm long x 10 mm ID with coarse frit.

5.2.3 Chromatographic column - 400 mm long x 19 mm ID with 250 mL reservoir at the top and Teflon stopcock (Kontes K-420290 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.8 Vials - Amber glass, 10 to 15-mL capacity with Teflon lined screw cap.

5.3 Boiling chips - Approximately 10/40 mesh. Heat to 400°C for 30 minutes or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Gas chromatograph - Analytical system complete with gas chromatograph suitable for splitless injection onto capillary columns and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas. Replacement of the glass injection port liner with a quartz liner is recommended but not required.

5.6.1 Column 1 - 30 m long x 0.25 mm fused silica capillary column coated with SE-54 (J&W Scientific or equivalent). This column was used to develop the method performance statements in Section 15. Guidelines for the use of alternate columns are provided in Section 12.1. The outlet end of the fused silica column should be threaded through the burner tip of the NPD to within 2 to 4 mm from the flame jet in order to minimize losses of anilines.

5.6.2 Column 2 - 180 cm long x 2 mm ID glass, packed with 3% SP-2250 on Supelcoport (100/120 mesh) or equivalent. Prior to use, column should be silylated by injecting a series of 8-10 injections (2 µL) of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Column should be held at 100°C with outlet end disconnected from the detector when performing these injections.

5.6.3 Column 3 - 30 m long x 0.25 mm fused silica capillary column coated with SE-30 (J&W Scientific or equivalent).

5.6.4 Detector - NPD or equivalent [i.e., Alkali-Flame Detector (AFD) or Thermionic Specific Detector (TSD)]. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 15.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the MDL of each parameter of interest.

6.2 Acetone, cyclohexane, hexane, isopropanol, methanol, methylene chloride, toluene - Pesticide quality or equivalent.

6.3 Sodium sulfate (ACS) Granular, anhydrous; heated in a muffle furnace at 400°C overnight.

6.4 Florisil - PR grade (60/100 mesh). Purchase activated at 1250°F and store in brown glass bottle. Extract with methylene chloride for 16 hours using a Soxhlet extractor, place 150 g in a widemouth brown bottle and heat overnight at 160-170°C. Seal tightly with Teflon or aluminum foil-lined cap and cool to room temperature. Isopropanol deactivation is accomplished in Section 11.2.1.

6.5 Stock standard solutions (1.00 µg/µL) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.5.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in pesticide quality toluene and dilute to volume in a 10 mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.5.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.5.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

6.6 BSTFA, reagent grade. Available from Pierce Chemical Company, Rockford, Illinois.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with toluene. One of the external standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 1 to 2 µL (2 to 5 µL for packed column) of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, linearity through the origin can be assumed and the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with toluene. One of the standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the expected range of concentrations found in real samples, or should define the working range of the detector.

7.3.2 Using injections of 1 to 2 µL (2 to 5 µL for packed column) of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_s C_{is})/A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard in µg/L.
C_s = Concentration of the parameter to be measured in µg/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 3, determine the average recovery (X) and standard deviation (P) expected for each method parameter, and compare the results to the calculated values for R and s. If $s>2P$ or $|X-R|>2P$, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts (7) that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly (7).

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices (8) should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. Fill the sample bottle, and at the time of collection if residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample. U.S. Environmental Protection Agency Methods 330.4 and 330.5 may be used for the measurement of residual chlorine (14). Field test kits are available for this purpose.

9.3 Adjust the pH of the sample to 6 to 8 with sodium hydroxide or sulfuric acid immediately after sampling.

9.4 All samples must be extracted within seven days and completely analyzed within 40 days of extraction.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel. Check the pH of the sample with wide range pH paper and adjust to pH $\geq$ 11 with 6 N sodium hydroxide.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner, again combining the extracts.

10.4 Assemble a Kuderna-Danish concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the Kuderna-Danish if the requirments of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the Kuderna-Danish concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.

10.6 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 ml methylene chloride to the top. Place the Kuderna-Danish apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the Kuderna-Danish apparatus and allow it to drain and cool for at least 10 minutes. Should it become necessary to discontinue the analytical procedure prior to the cleanup or solvent exchange step, the concentrated extract may be stored in the freezer at -10°C in the glass stoppered concentrator tube overnight. If the sample is to be analyzed without the cleanup step, proceed to Section 10.7. If the sample is to be cleaned up using adsorption chromatography, proceed to Section 11.

10.7 Increase the temperature of the hot water bath to 90-100°C. Momentarily remove the Snyder column, add 20 mL of cyclohexane and a new boiling chip and reattach the Snyder column. Pour about 1 mL of cyclohexane into the top of the Snyder column and concentrate the extract as before.

10.8 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of cyclohexane and add 0.5 mL of toluene. A 1.0-mL syringe is recommended for this operation. Add a new boiling chip, and attach a two-ball micro-Snyder column to the concentrator tube. Concentrate until an apparent volume of 0.5 mL is reached, or the solution stops boiling. Adjust the volume with toluene by quantitatively transferring to a 2.0-mL volumetric tube. If it is unnecessary to concentrate the sample to this extent, or the sample is not soluble in 2 mL, either a 5.0-mL or a 10-mL volumetric flask will suffice. If the extracts are to be stored longer than two days, transfer the sample from the volumetric glassware to a screw capped vial with a Teflon-lined cap.

10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 75%.

11.2 Florisil column cleanup for aniline compounds.

11.2.1 Fill a 400 mm x 19 mm ID chromatography column equipped with a 250 mL solvent reservoir with 100 mL of isopropanol/methylene chloride 5/95 (V/V) and add 20.0 g of activated Florisil.

11.2.2 Gently tap the column as the solvent is eluting to settle the chromatographic bed.

11.2.3 Wash the column with 100 mL of hexane/methylene chloride 50/50 (V/V), followed by 100 mL of hexane. Leave a column of about 5 cm of hexane above the Florisil.

11.2.4 Quantitatively transfer the sample extract onto 2.0 g of activated Florisil in a 50-mL beaker, using a small volume of methylene chloride, and dry under a gentle stream of nitrogen.

11.2.5 Place the dried Florisil containing the sample extract onto the chromatographic column, and wash the beaker which contained the Florisil with 75 mL of hexane, adding this wash to the reservoir. Elute the hexane fraction and discard. Stop the column flow just prior to the exposure of the Florisil to air.

11.2.6 Place a 500-mL Kuderna-Danish flask and clean concentrator tube under the chromatography column. Elute the column with 50 mL of 50% methylene chloride in hexane (V/V) (Fraction 1) using a drip rate of 5 mL/minute. Drain the column until the Florisil is nearly exposed to air then stop the column flow. If the fractions are to be collected separately, remove the Kuderna-Danish flask and set aside for later concentration. Elute the column again with 200 mL of 5% isopropanol in methylene chloride (V/V) (Fraction 2) into a second Kuderna-Danish flask. Elute the column a third time using 200 mL of 5% methanol in methylene chloride (V/V) (Fraction 3). The elution patterns for the aniline compounds are shown in Table 2. Frequently it will prove useful to combine the three fractions prior to analysis. However, in some situations analysis of each fraction separately may be required, e.g., to separately determine 2,6-dibromo-4-nitroaniline and 2,4-dinitroaniline using Column 3.

11.2.7 Concentrate the collected fractions to 2 mL final volume by Kuderna-Danish techniques as described in Section 10.6 through 10.8. Proceed with gas chromatographic analysis.

12. Gas Chromatography

Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method.

12.1 The SE-54 FSCC procedure is recommended for general use but will not resolve certain pairs of aniline compounds as indicated in Section 1.3 and Table 1. The packed column or alternate FSCC (SE-30) gas chromatographic method must be used for these materials. Alternate capillary columns may be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met. Packed columns should be silylated before use as described in Section 5.6.2.

12.2 If a capillary column is used for the analysis, the following performance check must be run on the column prior to using it in the method. Using the conditions specified for Column 1 in Table 1, inject 2 μL of a solution containing 10 μg/mL of each of the anilines. Adjust the detector sensitivity so that the later eluting compounds (e.g., 2,4-dinitroaniline) will be on scale. Just prior to the elution of 2-chloro-4,6-dinitroaniline increase the recorder chart speed to 5 cm/minute. After the 2,4-dinitroaniline peak has eluted, stop the recorder. Determine the asymmetry factor for 2,4-dinitroaniline as follows (10). This procedure is described in U.S. EPA Method 625 (11). Draw a perpendicular line from the apex of the peak to the baseline. Draw a horizontal line from the forward edge of the peak to the back edge of the peak at 10% of the peak height. Determine the distance from the front edge of the peak at 10% height to the intersection of the two lines (F). Determine the distance from the back edge of the peak at 10% height to the intersection of the two lines (B). The asymmetry factor (AF) is defined as:

$$AF = \frac{B}{F}$$

The AF for 2,4-dinitroaniline must be in the range of 0.9-3.0 in order for the column to be acceptable. The sum of B and F (total peak width at 10% height) should be less than 15 mm (at a chart speed of 5 cm/minute). Determine the separation number (SN) between 2-chloro-4,6-dinitroaniline (CDA) and 2,6-dibromo-4-nitroaniline (DBNA) using the following equation:

$$SN = \frac{D\ (DBNA - CNA)}{W_{1/2}(CDA) + W_{1/2}(DBNA)}$$

where $W_{1/2}(CDA)$ and $W_{1/2}(DBNA)$ are the widths at half height for for CDA and DBNA, respectively, and D (DBNA-CNA) is the distance between the apex of the two peaks. SN should be between 2 and 4 for Column 1. If Column 3 is used, only the asymmetry factor and peak width criteria for 2,4-dinitroaniline are appropriate due to differences in elution order between the two columns. DBNA must not be present in the mix used to test Column 3 since it coelutes wiht 2,4-dinitroaniline.

12.3 Calibrate the gas chromatographic system daily as described in Section 7.

12.4 If an internal standard approach is being used, add the internal standard to the sample extract and mix thoroughly prior to the transfer of the sample from any volumetric glassware.

12.5 Inject 2-µL or smaller volume of the sample extract using the splitless mode with a 45 second vent delay if using an automatic device. All sample extracts and standards must be dissolved in toluene when performing splitless FSCC analyses. If using a manual injection technique, use the solvent flush technique with an appropriate volume of sample extract such that the total volume of solvent injected does not exceed 2-µL (9). Record the volume injected to the nearest 0.05-µL, and the resulting peak sizes in area or peak height units.

12.6 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.7 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.8 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected in µL.
V_t = Volume of total extract in µL.
V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract in µg.
V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3., data for the affected parameters must be labeled as suspect.

14. GC-MS Confirmation

14.1 It is recommended that GC-MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound of interest. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 x per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all-glass or glass-lined materials is recommended. When using the fused silica SE-54 column, the column outlet should be threaded through the interface up to within a few mm of the entrance to the source ionization chamber. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chroamtographic program.

14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC-MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved. The calculation of tailing factors is illustrated in U.S. EPA Method 625 (11).

14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC-MS system must be checked to see that all DFTPP performance criteria are achieved (12).

14.4 To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 nanograms of material be injected into the GC-MS. The criteria below must be met for qualitative confirmation.

14.4.1 The molecular ion and all other ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to plus or minus 10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20-40%.

14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC-MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

14.7 GC-MS methodology for anilines has been evaluated in detail (1). The basic approach given in U.S. EPA Method 625 is appropriate with only minor modification. However, the use of a fused silica SE-54 column in place of the SP-2250 packed column is recommended, since this process eliminates the glass transfer line which can adsorb anilines if not carefully deactivated.

15. Method Performance

15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero (13). The MDL concentrations listed in Table 1 were obtained using reagent water (1). Similar results were achieved using representative wastewaters.

15.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from 3 x MDL to 300 x MDL with the following exceptions: 4-chloroaniline recovery was linear over the range 40-400 µg/L (40-400 x MDL). Aniline recovery was linear over the range 40-800 µg/L (16-320 x MDL).

13.5 In a single laboratory, Battelle Memorial Institute, using relevant spiked wastewater samples, the average recoveries presented in Table 3 were obtained. The standard deviation of the percent recovery is also included in Table 3 (1).

REFERENCES

1. Analytical Procedures for Aniline and Selected Derivatives in Wastewater and Sludge. Report for U.S. Environmental Protection Agency Contract Number 68-03-2952 (in preparation).

2. ASTM Annual Book of Standards, Part 31, D3694, Standard Practice for Preparation of Sample Containers and for Preservation, American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.

3. Carcinogens - Working with Carcinogens, Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August, 1977.

4. OSHA Safety and Health Standards, General Industry, (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised January, 1976).

5. Safety in Academic Chemistry Laboratories, American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

6. Interagency Testing Committee; Receipt of Fourth Report and Request for Comments, Federal Register, June 1, 1979, Vol. 44., pp. 31866-31889.

7. Handbook for Analytical Quality Control in Waste and Wastewater Laboratories, EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitroing and Support Laboratory-Cincinnati, Ohio 45268, March 1979.

8. ASTM Annual Book of Standards, Part 31, D3370, Standard Practice for Sampling Water, American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

9. Burke, J. A., Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects, Journal of the Association of Official Analytical Chemists, 48:1037 (1965).

10. McNair, H. M. and Bonelli, E. J., Basic Chromatography, Consolidated Printing, Berkley, CA, p. 52, 1969.

11. "Method 625 - Base/Neutrals and Acids", Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater. EPA-600/4-82-057, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1982.

12. Eichelberger, J. W., Harris, L. E., and Budde, W. L. Reference Compound to Calibrate Ion Abundance Measurement in Gas Chromatography-Mass Spectrometry, Analytical Chemistry, 47:995 (1975).

13. Glaser, J. A., Foerst, D. L., McKee, G. D., Quave, S. A., and Budde, W. L. Trace Analyses for Wastewaters. Env. Sci. Tech., 15(12): 1426-1435, 1981.

14. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual", Methods for Chemical Analysis of Water and Wastes. EPA 600/4-79-020, U.S. Environmental Protection Agency, Cincinnati, OH, 1979.

TABLE 1

GAS CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMIT

Parameter	Retention Time (min) Column 1	Column 2	Column 3	Method Detection Limit (µg/L)[a]
Aniline	7.5	8.6	6.3	2.3
2-Chloroaniline	12.1	11.5	7.1	1.4
3-Chloroaniline	14.6	14.8	9.0	1.8
4-Chloroaniline	14.7	15.2	9.1	0.66
4-Bromoaniline	18.0	18.6	12.1	4.6
2-Nitroaniline	21.9	23.4	15.6	1.0
2,4,6-Trichloroaniline	21.9	22.5	16.3	5.8
3,4-Dichloroaniline	22.7	20.0	16.6	3.2
3-Nitroaniline	24.5	26.1	18.0	3.3
2,4,5-Trichloroaniline	26.3	25.0	20.4	3.0
4-Nitroaniline	28.3	30.6	21.7	11
4-Chloro-2-nitroaniline	28.3	28.6	22.0	2.7
2-Chloro-4-nitroaniline	31.2	31.6	24.8	3.2
2,6-Dichloro-4-nitroaniline	31.9	31.2	26.0	2.9
2-Bromo-6-chloro-4-nitroaniline	34.8	34.0	28.8	3.4
2-Chloro-4,6-dinitroaniline	37.1	37.2	30.1	3.6
2,6-Dibromo-4-nitroaniline	37.6	36.9	31.6	3.8
2,4-Dinitroaniline	38.4	39.7	31.6	8.9
2-Bromo-4,6-dinitroaniline	39.8	40.2	33.4	3.7

Column 1 conditions: SE-54 Fused Silica 30 m x 0.25 mm column; with helium carrier gas at a flow of 28.5 cm/sec. at room temperature. Column temperature was held at 80°C for 4 min, programmed at 4°C/min to 230°C, then held at 230°C for 4 min.

Column 2 conditions: SP-2250 on Supelcoport (100/120 mesh) packed in a 180 cm x 2 mm ID glass column; with helium carrier gas at a flow of 30 mL/min at room temperature. Column program is the same as the capillary column.

Column 3 conditions: SE-30 Fused Silica 30 m x 0.25 mm column; with helium carrier gas at a flow of 30 cm/sec at room temperature. Column temperature was held at 80°C for 4 min, programmed at 4°C/min to 230°C, then held at 230°C for 4 min.

(a) MDL based upon seven replicate determinations in reagent water using Column 1.

TABLE 2

DISTRIBUTION OF ANILINES INTO FLORISIL COLUMN FRACTIONS

Parameter	Percent Recovery by Fraction		
	Fraction 1	Fraction 2	Fraction 3
Aniline		41	52
2-Chloroaniline		71	10
3-Chloroaniline		78	4
4-Chloroaniline	7	56	13
4-Bromoaniline		71	10
3,4-Dichloroaniline		83	1
2,4,6-Trichloroaniline(a)	70	14	
2,4,5-Trichloroaniline	35	53	
2-Nitroaniline(a)		97	9
3-Nitroaniline		97	11
4-Nitroaniline		67	30
2,4-Dinitroaniline(b)			75
4-Chloro-2-nitroaniline		84	
2-Chloro-4-nitroaniline		71	10
2,6-Dichloro-4-nitroaniline		89	9
2,6-Dibromo-4-nitroaniline(b)		89	9
2-Bromo-6-chloro-4-nitroaniline		88	16
2-Chloro-4,6-dinitroaniline			76
2-Bromo-4,6-dinitroaniline			106

(a) These compounds coelute on Column 1. If both compounds are to be determined the fractions should be analyzed individually.

(b) These compounds coelute on Column 3. If both compounds are to be determined (using Column 3) the fractions must be analyzed individually.

Eluate composition by fraction:
Fraction 1 - 50% methylene chloride in hexane
Fraction 2 - 5% Isopropanol in methylene chloride
Fraction 3 - 5% Methanol in methylene chloride

TABLE 3

SINGLE OPERATOR ACCURACY AND PRECISION

Parameter	Average Percent Recovery	Standard Deviation %	Spike Range (µg/L)	Number of Analyses	Matrix Types
Aniline	70	8.7	8.0-100	48	4
2-Chloroaniline	88	9.1	4.0-100	48	4
3-Chloroaniline	75	10	4.0-100	48	4
4-Chloroaniline	64	7.5	4.0-100	48	4
4-Bromoaniline	78	10	16.0-160	48	4
3,4-Dichloroaniline	79	13	8.0-100	48	4
2,4,6-Trichloroaniline	93	15	8.0-100	48	4
2,4,5-Trichloroaniline	85	12	8.0-100	48	4
2-Nitroaniline	92	14	4.0-100	48	4
3-Nitroaniline	80	11	8.0-100	48	4
4-Nitroaniline	94	21	8.0-100	48	4
2,4-Dinitroaniline	93	20	16.0-160	48	4
4-Chloro-2-nitroaniline	94	16	8.0-100	48	4
2-Chloro-4-nitroaniline	96	16	8.0-100	48	4
2,6-Dichloro-4-nitroaniline	92	12	8.0-100	48	4
2,6-Dibromo-4-nitroaniline	89	13	8.0-100	48	4
2-Bromo-6-chloro-4-nitroaniline	110	16	8.0-100	48	4
2-Chloro-4,6-dinitroaniline	92	17	8.0-100	48	4
2-Bromo-4,6-dinitroaniline	81	14	8.0-100	48	4

COLUMN: SE-54 FUSED SILICA
PROGRAM: 80°C 4 MIN., 4°C PER MIN. TO 230°C
DETECTOR: NPD

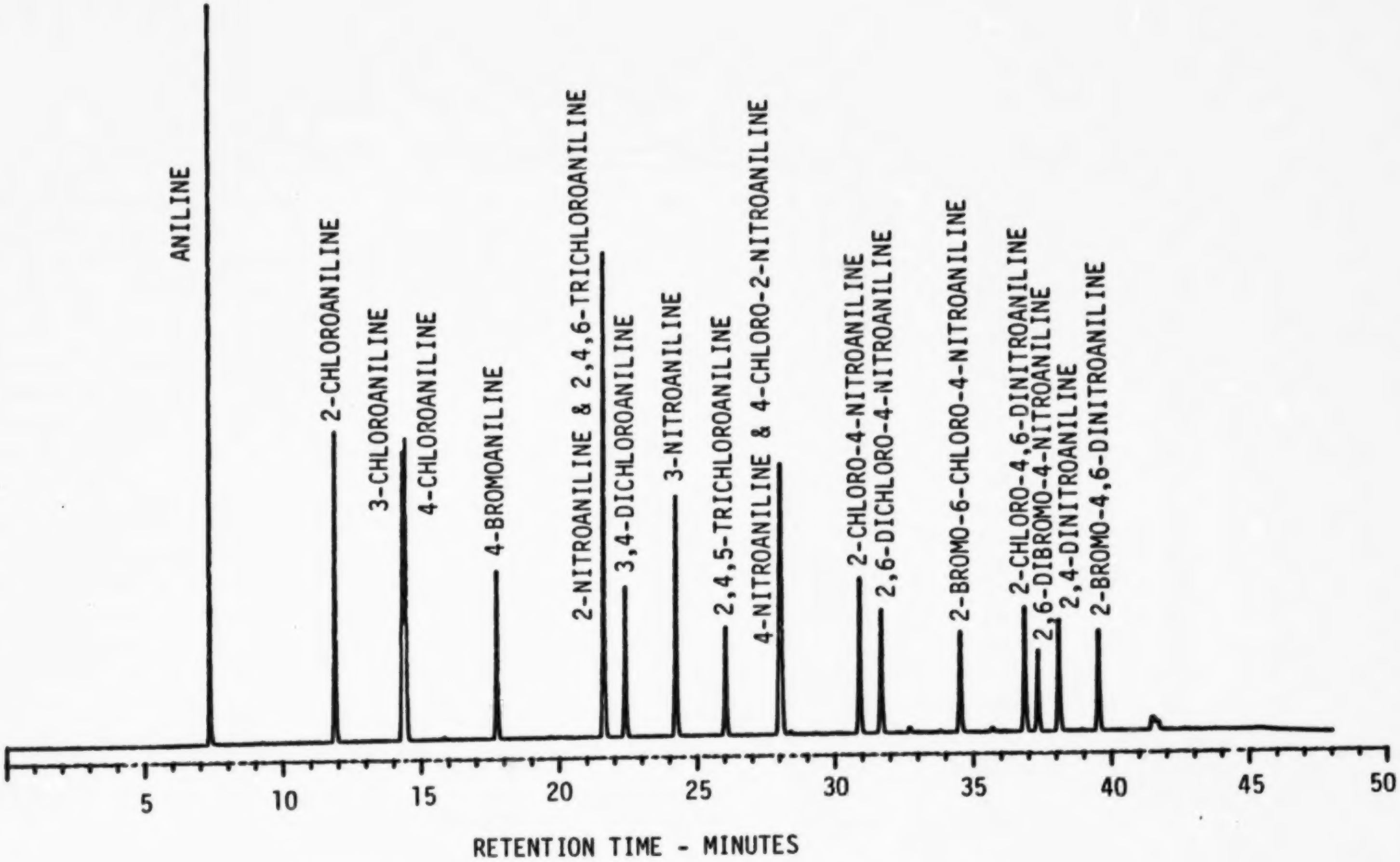

FIGURE 1. Gas chromatogram of aniline compounds on fused silica capillary column

COLUMN: 3% SP2250 ON SUPELCOPORT (100/120 MESH)
PROGRAM: 80°C 4 MIN., 4°C PER MIN. TO 230°C
DETECTOR: NPD

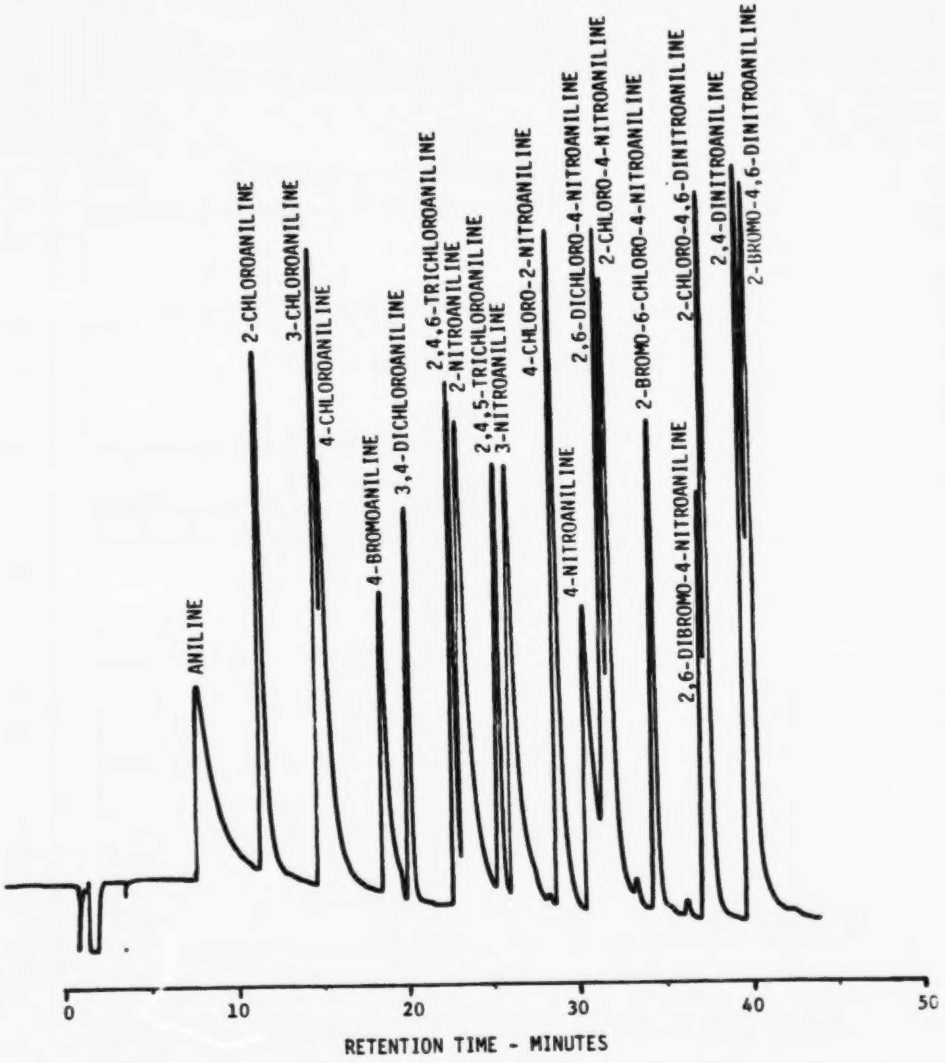

FIGURE 2. Gas chromatogram of aniline compounds on packed column

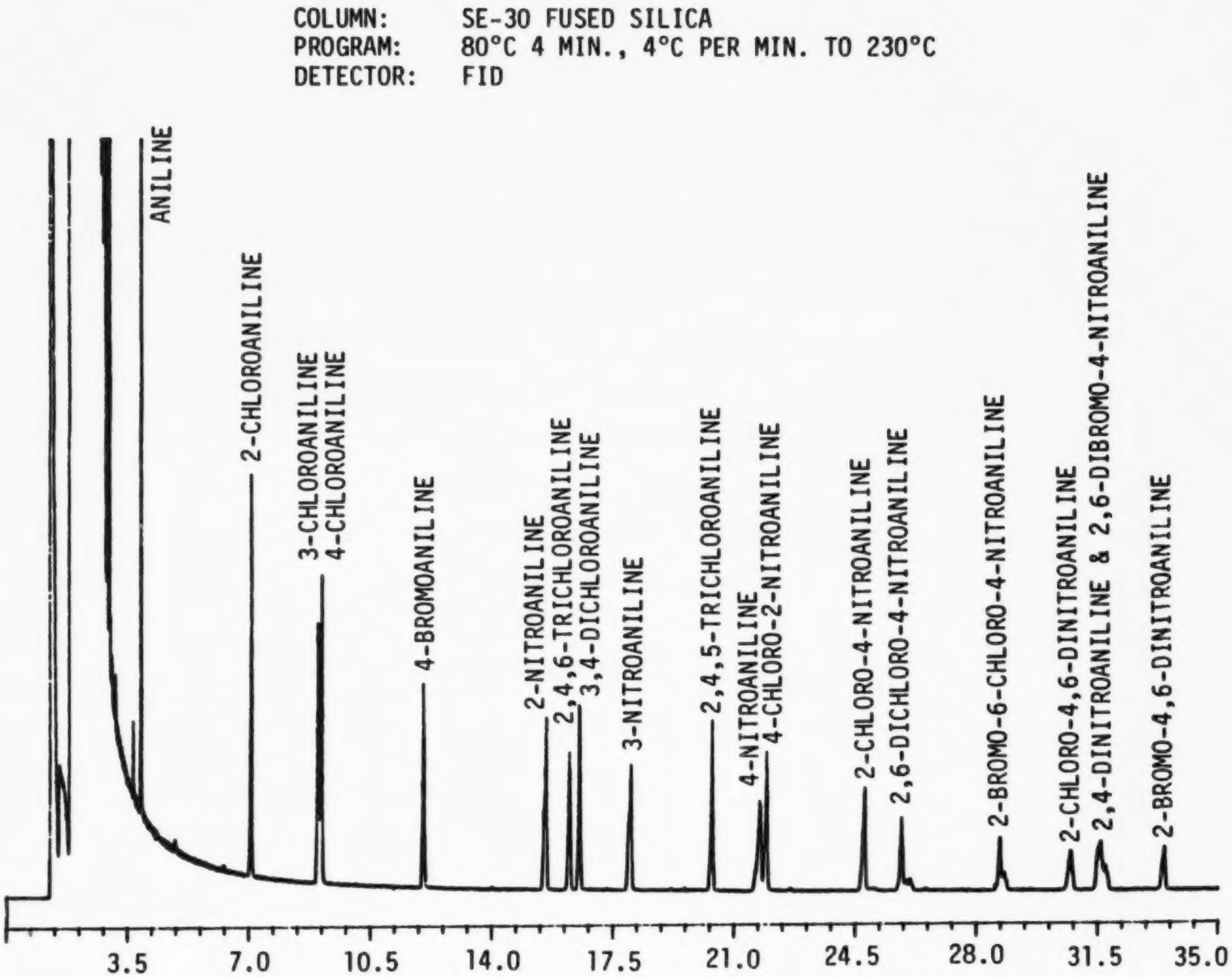

FIGURE 3. Gas chromatogram of aniline compounds on fused silica capillary column

APPENDIX B

DEFINITION AND PROCEDURE FOR THE DETERMINATION OF THE METHOD DETECTION LIMIT

DEFINITION

The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and determined from analysis of a sample in a given matrix containing analyte.

SCOPE AND APPLICATION

This procedure is designed for applicability to a wide variety of sample types ranging from reagent (blank) water containing analyte to wastewater containing analyte. The MDL for an analytical procedure may vary as a function of sample type. The procedure requires a complete, specific and well-defined analytical method. It is essential that all sample processing steps of the analytical method be included in the determination of the method detection limit.

The MDL obtained by this procedure is used to judge the significance of a single measurement of a future sample.

The MDL procedure was designed for applicability to a broad variety of physical and chemical methods. To accomplish this, the procedure was made device- or instrument-independent.

PROCEDURE

1. Make an estimate of the detection limit using one of the following:

    (a) The concentration value that corresponds to an instrument signal/noise in the range of 2.5 to 5.

    (b) Three times the standard deviation of replicate instrumental measurements of the reagent water.

    (c) The area of the standard curve where there is a significant change in sensitivity, i.e., a break in the slope of the standard curve.

    (d) Instrumental limitations.

It is recognized that the experience of the analyst is important to this process. However, the analyst must include the above considerations in the estimate of the detection limit.

2. Prepare reagent (blank) water that is as free of analyte as possible. Reagent or interference free water is defined as a water sample in which analyte and interference concentrations are not detected at the method detection limit of each analyte of interest. Interferences are defined as systematic errors in the measured analytical signal of an established procedure caused by the presence of interfering species (interference). The interference concentration is presupposed to be normally distributed in representative samples of a given matrix.

3. (a) If the MDL is to be determined in reagent (blank) water, prepare a laboratory standard (analyte in reagent water) at a concentration which is at least equal to or in the same concentration range as the estimated method detection limit. (Recommend between 1 and 5 times the estimated method detection limit.) Proceed to Step 4.

   (b) If the MDL is to be determined in another sample matrix, analyze the sample. If the measured level of the analyte is in the recommended range of one to five times the estimated detection limit, proceed to Step 4.

   If the measured level of analyte is less than the estimated detection limit, add a known amount of analyte to bring the level of analyte between one and five times the estimated detection limit.

   If the measured level of analyte is greater than five times the estimated detection limit, there are two options:

   (1) Obtain another sample containing a lower level of analyte in the same matrix if possible.

   (2) The untreated sample may be used for determining the method detection limit if the analyte level does not exceed 10 times the MDL of the analyte in reagent water. The variance of the analytical method changes as the analyte concentration increases from the MDL, hence the MDL determined under these circumstances may not truly reflect method variance at lower analyte concentrations.

4. (a) Take a minimum of seven aliquots of the sample to be used to calculate the method detection limit and process each aliquot through the entire analytical method. Make all computations

according to the defined method with final results in the method reporting units. If a blank measurement is required to calculate the measured level of analyte, obtain a separate blank measurement for each sample aliquot analyzed. The average blank measurement is subtracted from the respective sample measurements.

(b) It may be economically and technically desirable to evaluate the estimated method detection limit before proceeding with 4a. This step will: (1) prevent repeating this entire procedure when the costs of analyses are high, and (2) ensure that the procedure is being conducted at the correct concentration. It is quite possible that an incorrect MDL could be calculated from data obtained at many times the real MDL and the level of analyte would be less than five times the calculated method detection limit. To ensure that the estimate of the method detection limit is a good estimate, it is necessary to determine that a lower concentration of analyte will not result in a significantly lower method detection limit. Take two aliquots of the sample to be used to calculate the method detection limit and process each through the entire method, including blank measurements as described above in 4a. Evaluate these data:

(1) If these measurements indicate the sample is in desirable range for determination of the MDL, take five additional aliquots and proceed. Use all seven measurements for calculation of the MDL.

(2) If these measurements indicate the sample is not in correct range, reestimate the MDL, obtain new sample as in 3 and repeat either 4a or 4b.

5. Calculate the variance (S^2) and standard deviation (S) of the replicate measurements, as follows:

$$S^2 = \frac{1}{n - 1} \left\{ \left(\sum_{i=1}^{n} X_i^2 \right) - \left(\sum_{i=1}^{n} X_i \right)^2 \Big/ n \right\}$$

$$S = (S^2)^{1/2}$$

where the x_i, $i=1$ to n are the analytical results in the final method reporting units obtained from the n sample aliquots and refers to the sum of the X values from $i=1$ to n.

6. (a) Compute the MDL as follows:

$$MDL = t_{(n-1,.99)} \quad (S)$$

where:

MDL = the method detection limit

$t_{(n-1,.99)}$ = the student's t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. See table.

S = standard deviation of the replicate analyses.

(b) The 95% confidence interval estimates for the MDL derived in 6a are computed according to the following equations derived from percentiles of the chi square over degrees of freedom distribution (X^2/df).

$$LCL = 0.69 \text{ MDL}$$

$$UCL = 1.92 \text{ MDL}$$

where LCL and UCL are the lower and upper 95% confidence limits respectively, based on seven aliquots.

7. Optional iterative procedure to verify the reasonableness of the estimate of the MDL and subsequent MDL determinations.

(a) If this is the initial attempt to compute MDL based on the estimate of MDL formulated in Step 1, take the MDL as calculated in Step 6, spike in the matrix at the calculates MDL and proceed through the procedure starting with Step 4.

(b) If this is the second or later iteration of the MDL calculation, use S^2 from the current MDL calculation and S^2 from the previous MDL calculation to compute the F-ratio. The F-ratio is formed by substituting the largest S^2 of the two into the numerator S and the other into the denominator S. The computed F-ratio is then compared with the F-ratio found in the table which is 3.05 as follows:

$$\text{if} \quad \frac{S_A^2}{S_B^2} < 3.05,$$

then compute the pooled standard deviation by the following equation:

$$S_{pooled} = \left[\frac{6S_A^2 + 6S_B^2}{12}\right]^{1/2}$$

if $\frac{S_A^2}{S_B^2} > 3.05$, respike at the last calculated MDL and

process the samples through the procedure starting with Step 4.

(c) Use the S_{pooled} as calculated in Step 7b to compute the final MDL according to the following equation:

$$MDL = 2.681\ (S_{pooled})$$

where 2.681 is equal to $t_{(12, = .99)}$.

(d) The 95% confidence limits for MDL derived in Step 7c are computed according to the following equations derived from percentiles of the chi squared over degrees of freedom distribution.

$$LCL = 0.72\ MDL$$

$$UCL = 1.65\ MDL$$

where LCL and UCL are the lower and upper 95% confidence limits respectively, based on 14 aliquots.

TABLE OF STUDENT'S t VALUES AT THE 99 PERCENT CONFIDENCE LEVEL

Number of Replicates	Degrees of Freedom (n-1)	$t_{(n-1,.99)}$
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	0.764
16	15	2.602
21	20	2.528
26	25	2.485
31	30	2.457
61	60	2.390
00	00	2.326

REPORTING

The analytical method used must be specifically identified by number or title and the MDL for each analyte expressed in the appropriate method reporting units. If the analytical method permits options which affect the method detection limit, these conditions must be specified with the MDL value. The sample matrix used to determine the MDL must also be identified with MDL value. Report the mean analyte level with the MDL. If a laboratory standard or a sample that contained a known amount analyte was used for this determination, also report the mean recovery.

If the level in the sample was below the determined MDL or does not exceed 10 times the MDL of the analyte in reagent water, do not report a value for the MDL.

APPENDIX C

PROCEDURE FOR DETERMINATION OF THE ANALYTICAL CURVE

1. The analyst shall establish the upper limit of the operating range from the following:

   (a) An estimate of the upper limit is to be made from the method detection limit (MDL) by using the factor 1000 x MDL or 100 µg/L (whichever is larger) to estimate the concentration.

   (b) In the case where the solubility of an analyte is exceeded by the options listed in part 1(a), the maximum solubility is to be used as an estimate of the upper limit of the operating range.

2. The analyst shall sample across the operating range duplicate points at 10x, 100x, 1000x, or multiples thereof, depending on the upper limit of the operating range as established in step 1 and then use a linear least squares regression to fit the points. Where 100 µg/L or solubility is used to estimate the upper limit of the operating range, the ratio of 100 µg/L/MDL or solubility/MDL is to be used to pick three equal ranges which would correspond to the sampling scheme of three orders of ten as described above. Depending on the width of the analytical curve fitting the above restrictions, the sampling points may either be expanded or contracted to cover the range.

3. Based on these results, four additional points will be run at concentrations to be determined by consultation with the project officer.

4. A total of ten points are to be collected for the determination of the analytical curve.

APPENDIX D

MASS SPECTRA FOR ANILINE COMPOUNDS

TABLE 1. RELATIVE ION ABUNDANCES FOR ANILINE USING CAPILLARY COLUMN GC-MS AND 4-CHLOROANILINE USING PACKED COLUMN GC-MS

Aniline		4-Chloroaniline	
M/E	Relative Abundance %	M/E	Relative Abundance %
40	5.77	40	3.92
41	5.47	41	7.76
47	4.82	44	2.46
50	4.19	45	6.22
51	4.66	49	3.27
52	5.45	50	6.42
54	4.50	51	4.98
61	2.00	52	8.92
62	2.95	53	2.10
63	5.28	54	2.34
64	3.39	61	6.37
65	21.96	62	8.55
66	44.13	63	17.73
67	5.60	64	12.25
78	2.14	65	38.02
92	13.07	66	5.19
93	100.00	73	8.18
94	6.72	74	3.75
		75	4.64
		90	3.71
		91	7.57
		92	22.41
		93	2.18
		99	8.31
		100	15.14
		101	4.10
		102	4.93
		127	100.00
		128	7.46
		129	31.49
		130	2.54

TABLE 2. RELATIVE ION ABUNDANCES FOR 2-CHLOROANILINE AND 3-CHLOROANILINE USING PACKED COLUMN GC-MS

2-Chloroaniline		3-Chloroaniline	
M/E	Relative Abundance %	M/E	Relative Abundance %
40	2.89	40	3.50
41	6.63	41	7.12
42	2.05	42	2.43
45	6.48	45	6.75
49	2.86	49	2.98
50	5.42	50	5.88
51	5.10	51	4.90
52	13.43	52	9.93
61	5.58	54	2.19
62	7.85	61	6.58
63	18.67	62	8.51
46	18.29	63	17.99
65	35.01	64	13.82
66	4.51	65	40.99
73	6.22	66	5.59
74	3.37	73	5.69
75	4.07	74	2.89
90	4.26	75	4.46
91	9.97	90	4.03
92	23.09	91	8.52
99	6.48	92	24.50
100	10.34	99	7.01
101	3.15	100	14.14
102	3.64	101	3.62
127	100.00	102	4.99
128	7.54	127	100.00
129	31.73	128	7.63
130	2.30	129	31.37
		130	2.21

TABLE 3. RELATIVE ION ABUNDANCES FOR 4-BROMOANILINE USING PACKED COLUMN GC-MS

M/E	Relative Abundance %	M/E	Relative Abundance %
40	6.72	76	2.50
41	13.33	79	7.12
42	3.48	80	4.08
44	3.44	81	5.78
45	6.67	82	4.36
46	5.37	85	2.94
49	3.94	86	3.69
50	12.14	87	3.29
51	7.01	90	6.66
52	16.10	91	12.35
53	2.65	92	77.74
54	4.92	93	6.44
60	2.32	117	3.94
61	9.12	110	4.83
62	14.70	127	10.00
63	30.31	129	3.45
64	16.05	143	3.70
65	98.12	145	4.02
66	11.41	171	100.00
73	2.94	172	8.63
74	5.91	173	97.77
75	3.21	174	7.32

TABLE 4. RELATIVE ION ABUNDANCES FOR 2-NITROANILINE & 3-NITROANILINE USING PACKED COLUMN GC-MS

2-Nitroaniline		3-Nitroaniline	
M/E	Relative Abundance %	M/E	Relative Abundance %
40	5.67	40	5.80
41	8.48	41	10.59
42	3.46	42	3.96
44	3.89	44	6.26
46	8.61	46	11.87
49	4.45	50	5.56
50	13.02	51	5.89
51	16.90	52	16.23
52	31.38	53	8.99
53	13.65	54	4.20
54	4.40	61	5.04
61	7.64	62	8.02
62	14.11	63	17.32
63	42.26	64	13.52
64	31.80	65	100.00
65	100.00	66	13.65
66	13.53	74	3.86
74	5.19	75	2.13
75	5.62	80	25.74
76	4.63	81	2.68
77	10.25	90	2.26
78	3.98	91	4.89
80	39.07	92	73.83
88	2.43	93	7.17
90	40.37	108	10.70
91	13.08	110	2.55
92	56.03	122	3.30
93	5.16	138	66.95
104	4.05	139	5.67
108	34.22		
109	2.94		
120	28.07		
121	8.82		
122	5.18		
138	92.79		
139	7.20		

TABLE 5. RELATIVE ION ABUNDANCES FOR 4-NITROANILINE & 3,4-DICHLOROANILINE USING PACKED COLUMN GC-MS

4-Nitroaniline		3,4-Dichloroaniline	
M/E	Relative Abundance %	M/E	Relative Abundance %
40	5.67	40	3.14
41	13.21	41	10.41
42	4.20	44	5.38
44	5.88	47	3.08
46	10.62	49	4.70
50	8.17	50	5.58
51	8.02	51	3.71
52	20.20	52	9.78
53	15.42	60	4.00
54	6.91	61	11.29
61	4.81	62	18.54
62	9.71	63	30.78
63	19.37	64	8.15
64	13.45	65	6.13
65	100.00	72	6.46
66	13.12	73	11.05
74	3.23	74	6.07
75	2.18	75	5.26
77	2.20	82	3.23
78	2.13	89	4.10
79	3.16	90	28.69
80	35.67	91	7.67
81	2.77	97	4.57
91	4.66	99	30.17
92	42.38	100	3.45
93	3.68	101	9.21
105	2.21	125	10.44
108	89.68	126	17.53
109	6.70	127	4.61
122	7.63	128	6.08
138	79.68	133	4.54
139	6.31	134	8.06
		135	3.54
		136	5.14
		161	100.00
		162	5.18
		163	65.44
		164	4.31
		165	10.52

TABLE 6. RELATIVE ION ABUNDANCES FOR
2,4-DINITROANILINE
USING PACKED COLUMN GC-MS

M/E	Relative Abundance %	M/E	Relative Abundance %
40	13.53	79	25.93
41	21.85	80	13.19
44	30.73	87	9.69
46	21.90	88	17.58
49	7.64	89	20.44
50	18.31	90	10.62
51	27.14	91	68.47
52	83.71	92	13.99
53	17.94	93	7.60
54	7.20	95	8.98
61	25.93	105	25.77
62	59.44	107	42.82
63	62.10	119	8.41
64	63.23	135	5.04
65	20.46	137	11.92
66	12.50	153	76.85
67	7.97	154	7.36
75	13.02	165	38.43
76	13.87	166	5.84
77	37.10	167	8.81
78	24.35	183	100.00
		184	7.53

TABLE 7. RELATIVE ION ABUNDANCES FOR 2,4,5-TRICHLOROANILINE USING PACKED COLUMN GC-MS

M/E	Relative Abundance %	M/E	Relative Abundance %
41	3.41	97	25.00
44	4.71	98	9.55
47	6.11	99	13.30
48	2.79	100	3.22
49	6.40	107	6.82
50	4.71	108	2.05
51	4.08	109	4.84
52	16.35	123	2.29
53	2.33	124	29.97
60	7.87	125	9.46
61	19.61	126	9.70
62	26.31	127	3.05
63	16.63	132	5.28
64	3.56	133	25.00
65	5.62	134	3.83
71	3.38	135	17.28
72	9.51	137	3.93
73	10.07	159	9.55
74	7.05	160	15.53
75	7.27	161	7.36
76	3.76	162	9.94
79	3.32	167	5.12
80	4.20	168	2.11
83	3.55	169	4.85
84	4.06	170	2.15
85	2.39	195	100.00
86	5.67	196	8.00
87	3.72	197	95.68
88	8.77	198	7.05
89	7.95	199	31.19
90	7.33	200	2.31
96	5.58	201	3.59

TABLE 8. RELATIVE ION ABUNDANCES FOR
2,4,6-TRICHLOROANILINE
USING PACKED COLUMN GC-MS

M/E	Relative Abundance %	M/E	Relative Abundance %
47	5.00	107	7.53
48	2.84	109	5.75
49	5.94	110	1.37
50	3.62	123	7.25
51	2.69	124	50.06
52	13.49	125	10.84
60	5.94	126	15.86
61	19.52	127	2.66
62	26.08	131	2.61
63	15.26	132	6.34
64	2.12	133	15.89
71	3.36	134	5.44
72	8.04	135	10.30
73	12.25	137	2.52
74	6.69	158	1.19
75	7.24	159	19.86
76	3.73	160	15.23
81	4.24	161	12.89
83	3.28	163	9.28
84	2.32	164	2.33
85	3.37	165	1.24
86	4.84	167	3.88
87	3.92	168	1.84
88	19.18	169	3.63
89	9.67	170	1.62
90	8.26	171	1.20
95	1.96	195	100.00
96	5.15	196	4.76
97	30.76	197	96.78
98	9.31	198	4.36
99	14.99	199	30.95
100	2.03	200	1.68
101	1.09	201	2.76

TABLE 9. RELATIVE ION ABUNDANCES FOR
2-BROMO-6-CHLORO-4-NITROANILINE
USING PACKED COLUMN GC-MS

M/E	Relative Abundance %	M/E	Relative Abundance %
44	7.34	124	71.97
46	19.06	125	38.13
49	5.66	126	27.33
50	8.57	127	13.48
51	10.83	141	13.39
52	43.39	142	7.91
53	6.38	160	5.45
60	7.60	162	5.06
61	32.19	168	26.96
62	47.61	169	5.98
63	44.31	170	24.39
64	5.84	171	5.22
65	14.53	176	10.36
73	10.79	177	13.63
74	8.63	178	17.90
75	11.98	181	5.48
76	10.13	192	14.29
77	6.98	194	18.33
78	13.07	196	5.16
80	13.92	204	28.64
81	11.11	205	5.80
86	7.25	206	42.41
87	7.45	208	12.13
88	13.59	220	70.87
89	31.33	222	100.00
90	54.16	223	8.49
95	5.16	224	23.10
97	20.87	234	5.00
98	9.53	250	67.07
99	9.72	251	5.98
113	7.74	252	89.72
114	7.51	253	7.10
117	5.72	254	23.87

TABLE 10. RELATIVE ION ABUNDANCES FOR 2-BROMO-4,6-DINITROANILINE USING PACKED COLUMN GC-MS

M/E	Relative Abundance %	M/E	Relative Abundance %
44	38.04	141	10.42
46	27.27	142	14.98
49	6.58	143	12.44
50	17.29	144	13.29
51	15.33	153	5.77
52	40.69	154	5.04
53	10.27	155	6.48
61	39.46	156	6.60
62	57.69	157	8.52
63	70.08	158	6.08
64	14.08	159	6.54
65	16.65	169	38.35
67	5.83	170	10.31
74	8.29	171	34.77
75	9.46	172	7.29
76	17.04	173	8.10
77	13.02	175	7.37
78	43.46	181	5.73
79	12.54	183	5.02
80	15.23	185	28.15
81	7.10	187	28.81
82	10.33	188	5.19
87	16.13	199	8.96
88	37.04	201	6.65
89	30.00	215	10.67
90	100.00	217	14.71
91	13.92	231	48.15
92	9.38	232	6.29
94	9.29	233	48.31
98	6.17	234	5.25
105	6.60	243	11.94
106	9.38	245	14.67
111	5.40	247	7.02
118	9.33	261	88.62
119	10.96	262	7.92
120	8.87	263	90.62
124	6.33	264	9.81
125	5.15		

TABLE 11. RELATIVE ION ABUNDANCES FOR 2,6-DICHLORO-4-NITROANILINE USING PACKED COLUMN GC-MS

M/E	Relative Abundance %	M/E	Relative Abundance %
41	3.22	109	3.02
44	8.13	112	7.08
46	17.62	113	3.63
47	4.63	114	4.72
49	6.79	121	3.05
50	7.47	124	100.00
51	10.10	125	20.26
52	35.79	126	32.18
53	5.13	127	[illegible].59
60	6.50	133	25.65
61	24.27	135	17.12
62	36.55	137	3.70
63	29.77	140	5.85
65	12.65	148	18.98
72	7.09	150	14.53
73	19.73	152	3.09
74	9.44	159	4.20
75	14.08	160	39.96
76	12.11	161	3.12
77	5.62	162	27.36
78	6.93	164	4.76
85	6.64	176	85.44
86	8.20	177	3.22
87	5.26	178	57.13
88	13.58	179	6.24
89	20.41	180	7.19
90	28.71	190	3.88
96	4.55	206	68.27
97	28.31	207	5.06
98	9.08	208	43.12
99	11.85	209	3.12
100	4.78	210	7.78
107	3.77		

TABLE 12. RELATIVE ION ABUNDANCES FOR 2-CHLORO-4,6-DINITROANILINE USING PACKED COLUMN GC-MS

M/E	Relative Abundance %	M/E	Relative Abundance %
40	4.36	97	14.41
41	4.73	98	31.23
43	7.13	99	6.64
44	17.53	100	18.16
46	27.61	101	7.76
47	5.04	104	4.62
49	10.81	106	4.57
50	15.50	111	14.14
51	18.16	112	6.33
52	49.53	113	22.32
53	9.47	114	7.31
60	6.80	115	7.58
61	34.38	119	4.36
62	51.41	123	6.49
63	52.28	125	88.87
64	13.94	126	11.41
65	19.00	127	29.62
69	4.34	128	5.91
72	8.50	129	13.84
73	15.63	131	6.68
74	13.97	139	6.23
75	17.83	141	54.36
76	22.42	142	4.62
77	10.72	143	19.03
78	41.89	153	4.59
79	4.23	171	14.71
80	5.76	173	6.39
85	6.20	187	62.47
86	13.47	189	22.39
87	17.33	199	15.25
88	44.37	201	11.71
89	35.12	217	100.00
90	77.75	218	9.60
91	9.57	219	33.91
94	5.95	220	4.04
96	4.25		

TABLE 13. RELATIVE ION ABUNDANCES FOR
4-CHLORO-2-NITROANILINE
USING PACKED COLUMN GC-MS

M/E	Relative Abundance %	M/E	Relative Abundance %
40	4.85	89	9.33
41	10.09	90	63.96
44	7.30	91	21.81
46	13.09	92	3.39
47	6.01	97	15.33
49	18.09	98	11.28
50	19.02	99	64.42
51	22.99	100	9.91
52	35.45	101	20.16
53	6.82	103	7.87
54	3.21	112	3.71
60	7.11	114	31.71
61	24.77	116	12.75
62	40.05	124	69.07
63	75.18	125	10.33
64	32.66	126	98.72
65	13.75	127	8.30
66	4.19	128	26.46
72	7.74	142	26.55
73	38.32	144	9.56
74	15.21	154	43.29
75	22.97	155	4.87
76	16.61	156	20.14
77	3.95	517	3.06
78	12.20	172	100.00
84	4.15	173	5.26
86	6.23	174	34.95
87	10.30	175	3.19
88	15.15		

TABLE 14. RELATIVE ION ABUNDANCES FOR 2-CHLORO-4-NITROANILINE USING PACKED COLUMN GC-MS

M/E	Relative Abundance %	M/E	Relative Abundance %
40	4.04	87	4.74
41	11.31	88	5.33
42	2.36	89	6.79
44	3.90	90	100.00
46	14.32	91	16.06
49	3.99	97	4.20
50	6.17	99	31.15
51	13.20	100	3.18
52	36.23	101	11.38
53	5.81	105	3.26
54	3.09	106	3.52
60	3.39	114	24.63
61	11.46	116	9.07
62	21.88	126	27.91
63	58.51	127	2.99
64	17.07	128	9.42
65	12.07	142	89.72
66	3.16	143	3.66
72	3.05	144	30.97
73	15.88	145	2.36
74	5.94	156	4.63
75	9.20	158	2.33
76	4.32	172	75.64
78	8.35	173	4.09
79	4.05	174	26.04
80	5.10	175	2.41
86	3.26		

TABLE 15. RELATIVE ION ABUNDANCES FOR 2,6-DIBROMO-4-NITROANILINE USING PACKED COLUMN GC-MS

M/E	Relative Abundance %	M/E	Relative Abundance %	M/E	Relative Abundance %
40	4.71	96	5.39	170	64.30
41	5.60	98	4.97	171	26.44
44	12.37	104	6.39	172	4.92
45	7.96	105	6.39	176	14.84
46	24.58	106	6.64	178	10.46
50	15.85	114	4.07	185	6.07
51	20.23	115	4.14	187	4.12
52	63.01	116	4.03	206	14.96
53	10.59	117	7.85	208	8.28
60	9.91	118	5.77	220	8.26
61	53.56	119	9.70	221	7.05
62	79.58	121	4.25	223	10.33
63	87.90	124	24.02	225	6.03
64	10.61	125	8.72	236	7.98
65	17.02	126	8.25	237	8.42
73	10.72	127	6.60	238	6.26
74	10.51	130	4.90	239	10.84
75	8.28	131	4.58	248	16.79
76	14.60	133	8.15	250	35.21
77	9.74	135	5.35	251	4.82
78	22.54	141	14.84	252	19.78
79	20.42	142	11.04	264	53.48
80	25.00	143	11.27	265	4.41
81	17.70	144	6.88	266	96.52
82	26.21	148	4.12	267	8.83
85	6.83	156	6.13	268	50.68
86	9.04	158	9.17	269	4.54
88	23.64	159	6.94	280	5.30
89	50.76	160	7.89	294	40.85
90	100.00	162	6.07	295	4.71
91	11.06	168	63.69	296	84.42
93	5.37	169	26.82	298	42.66

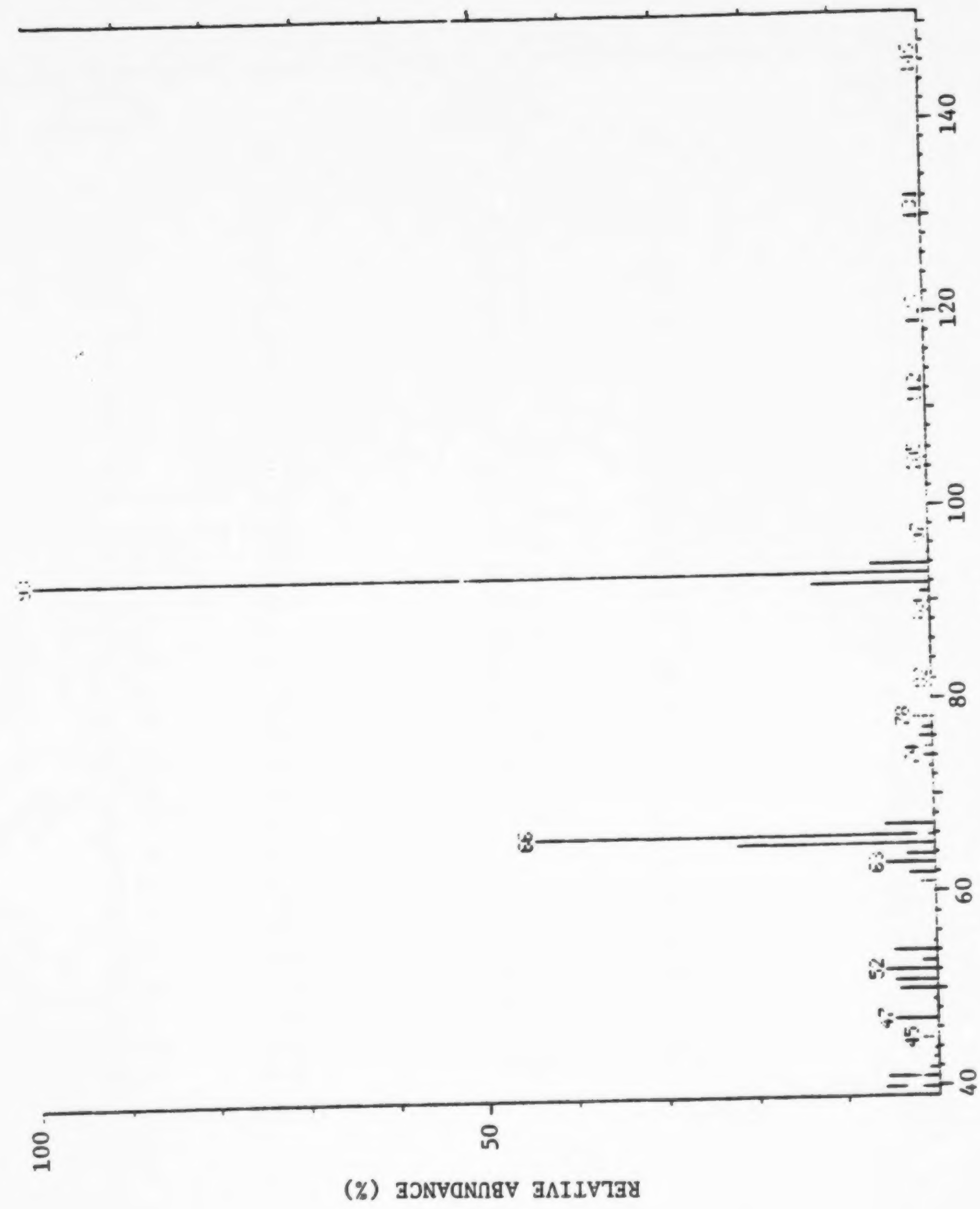

FIGURE D-1. Mass spectrum for Aniline using capillary column GC-MS.

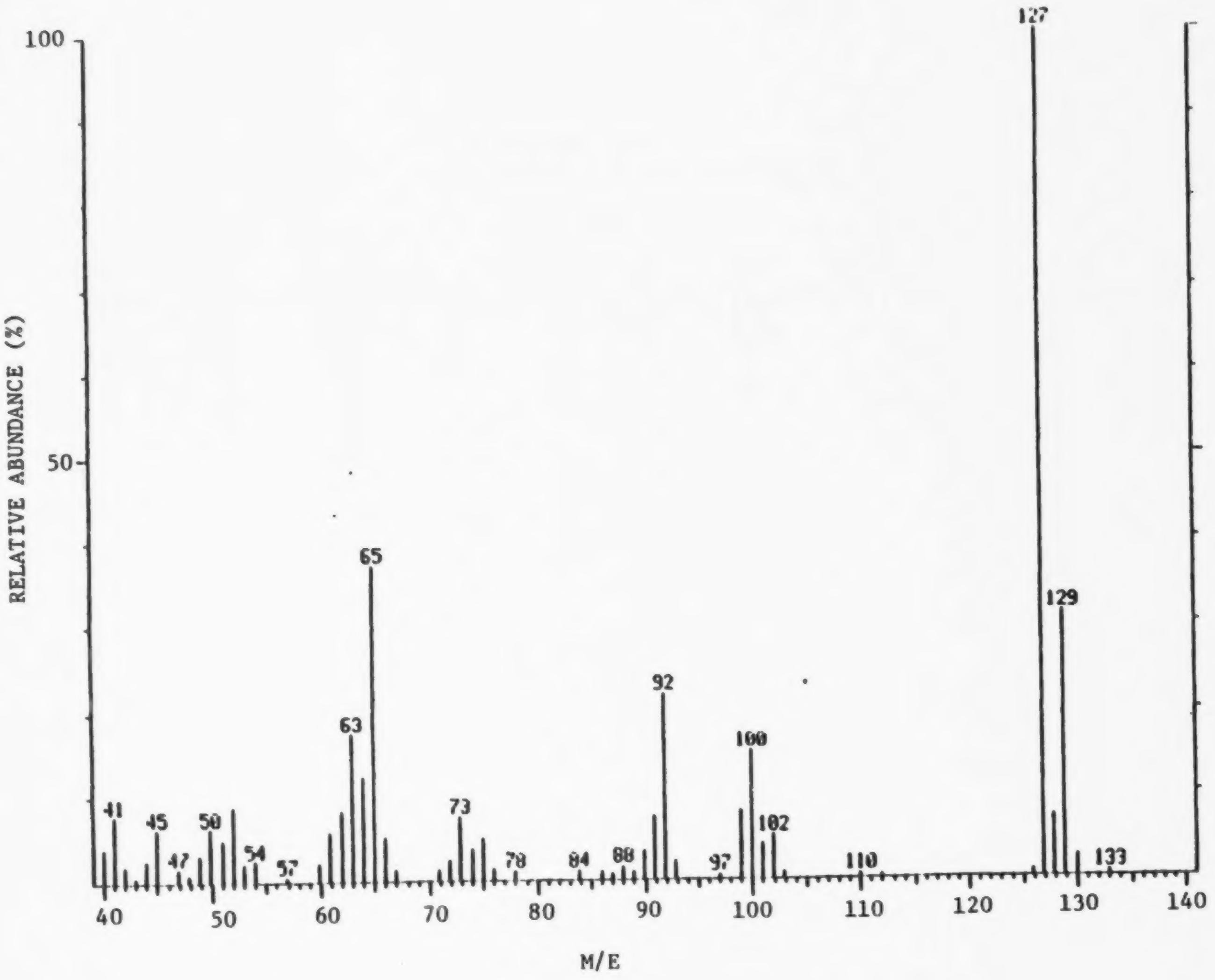

FIGURE D-2. Mass spectrum for 4-Chloroaniline using packed column GC-MS.

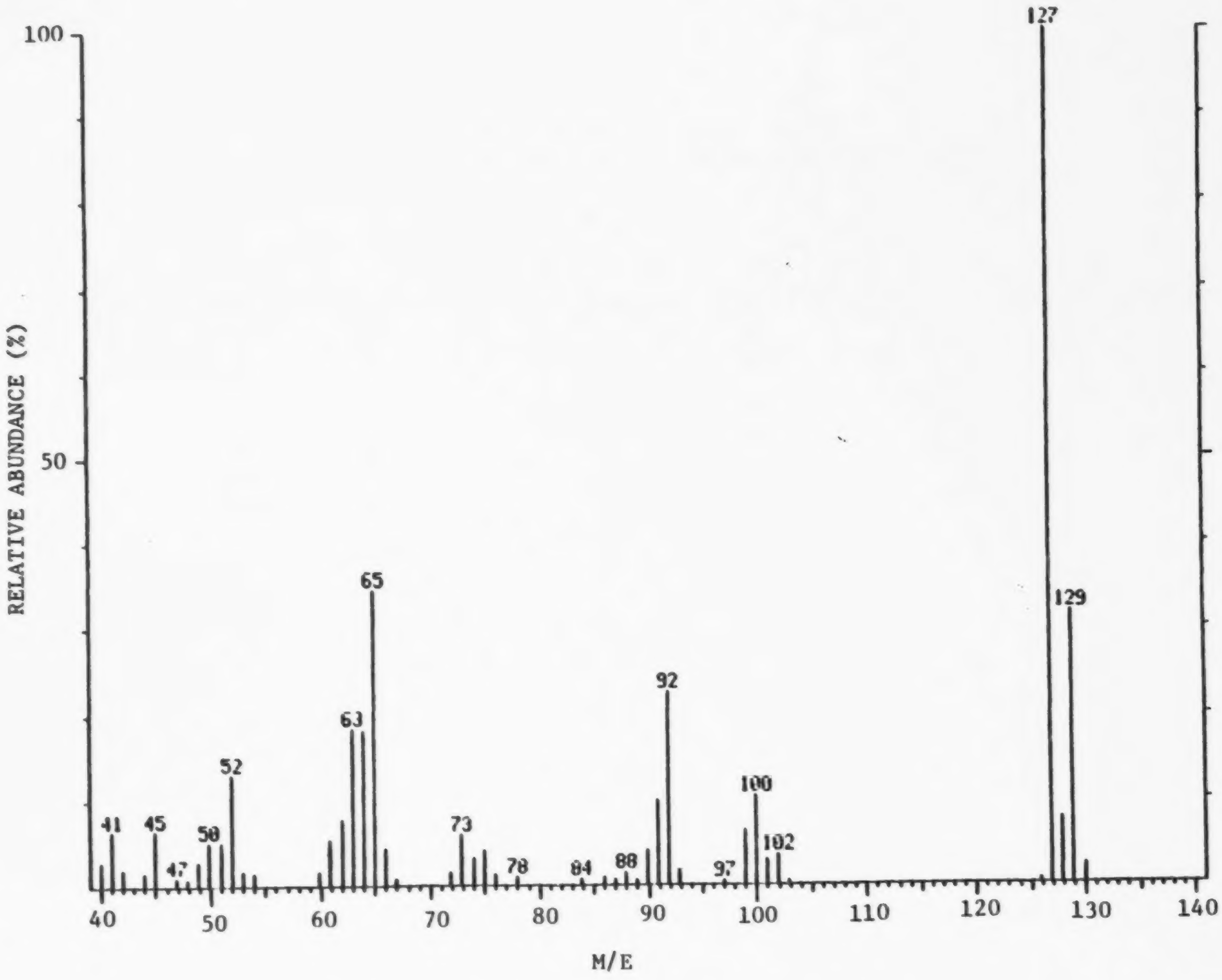

FIGURE D-3. Mass spectrum for 2-Chloroaniline using packed column GC-MS.

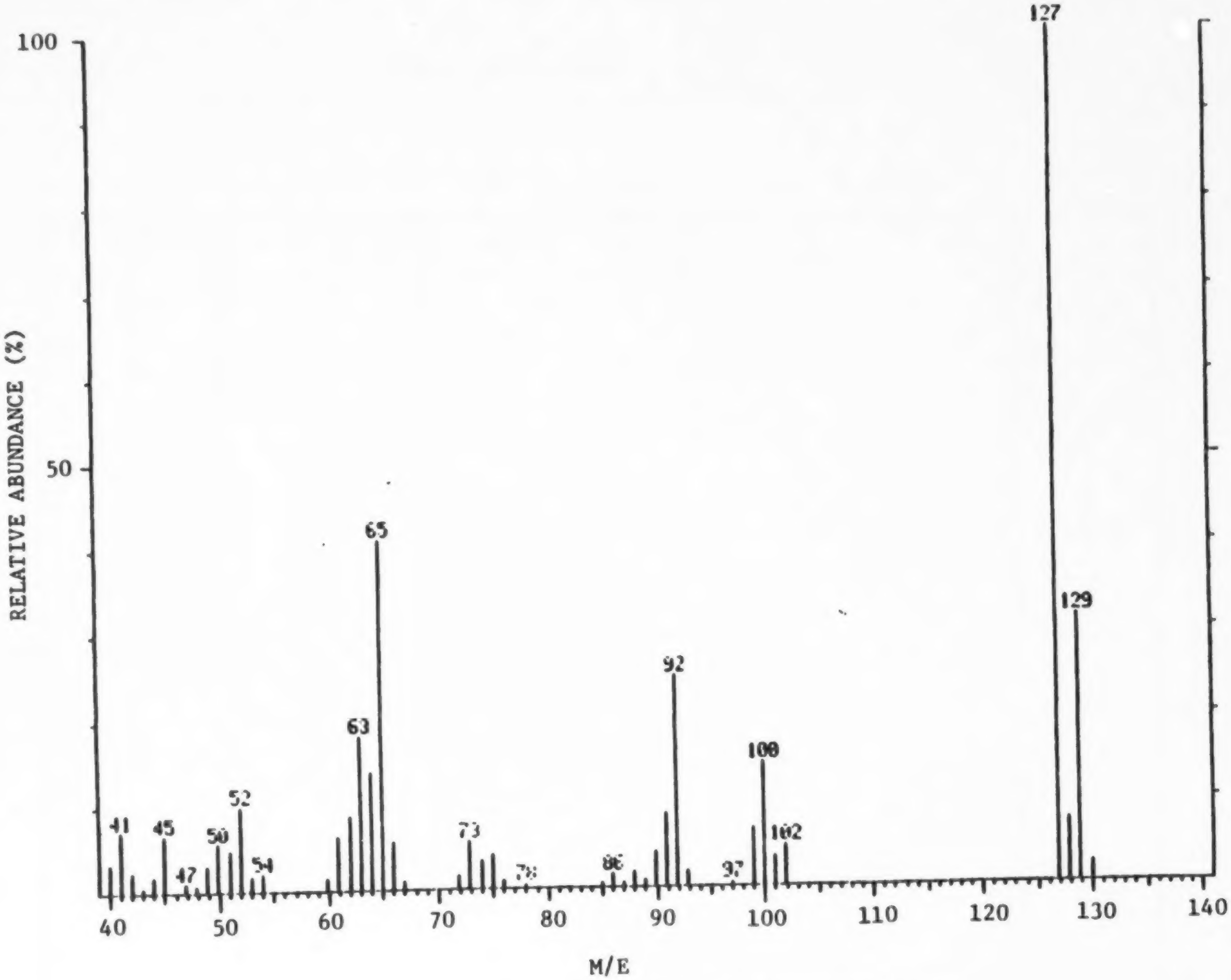

FIGURE D-4. Mass spectrum for 3-Chloroaniline using packed column GC-MS.

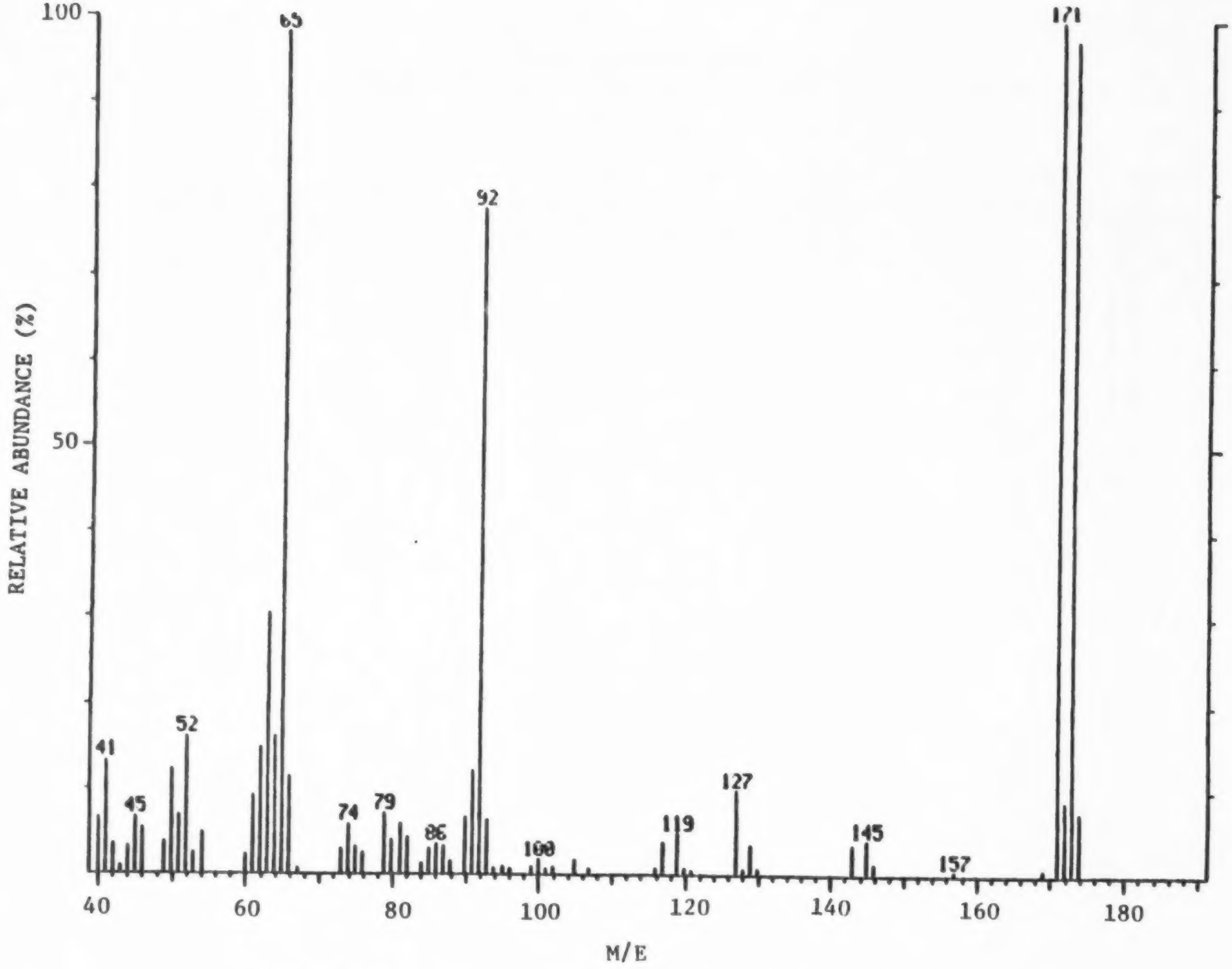

FIGURE D-5. Mass spectrum for 4-Bromoaniline using packed column GC-MS.

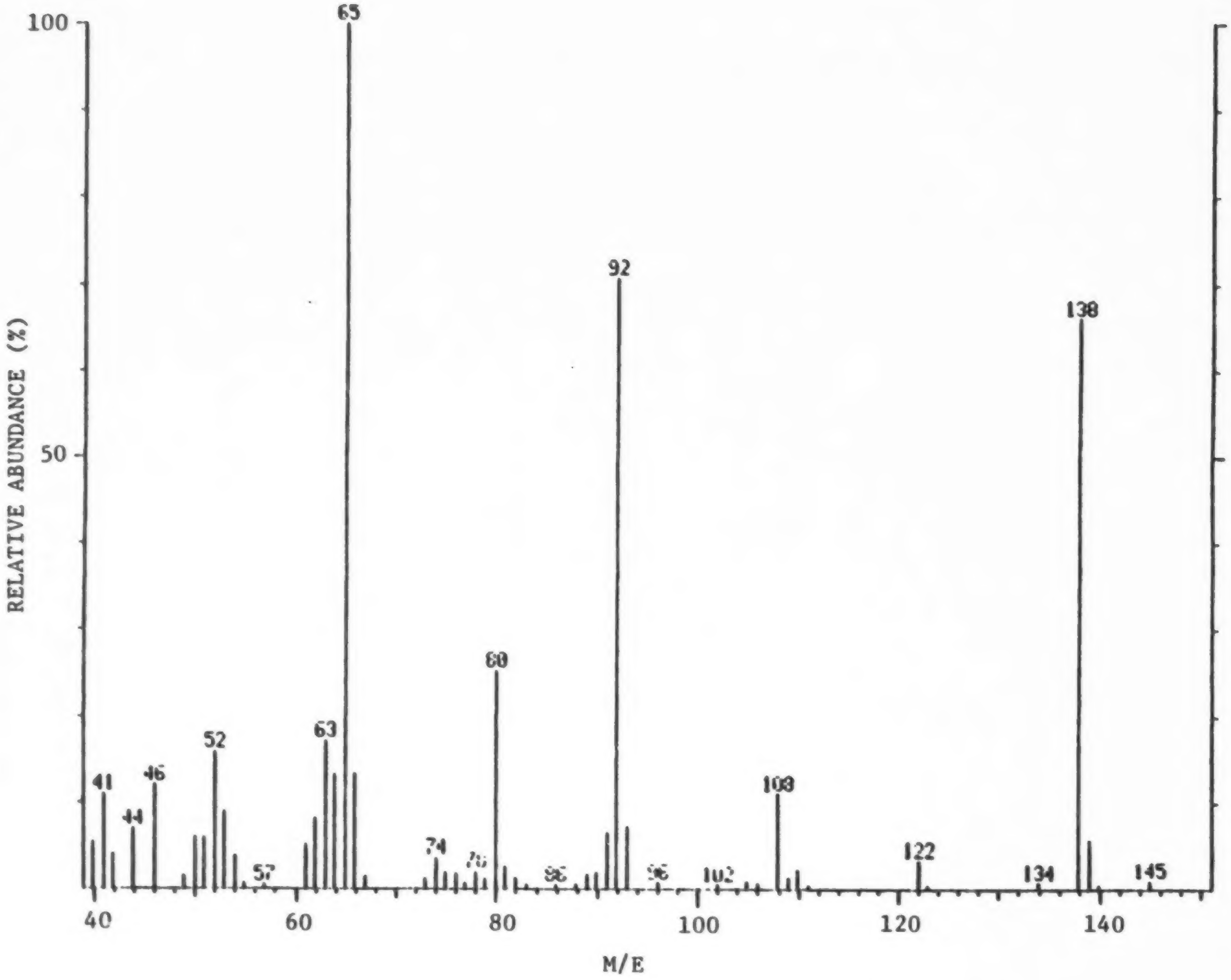

FIGURE D-6. Mass spectrum for 3-Nitroaniline using packed column GC-MS.

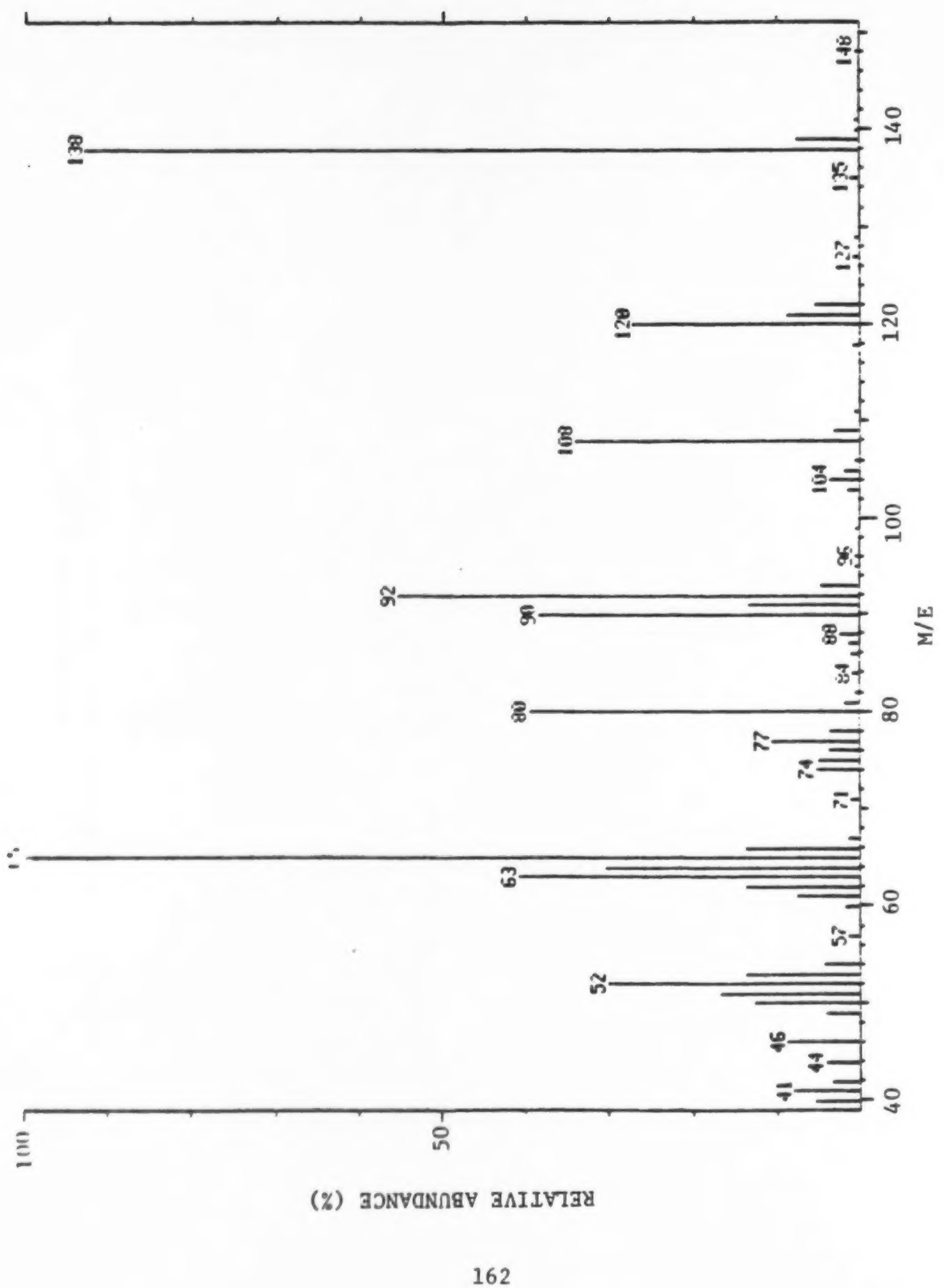

FIGURE D-7. Mass spectrum for 2-Nitroaniline using packed column GC-MS.

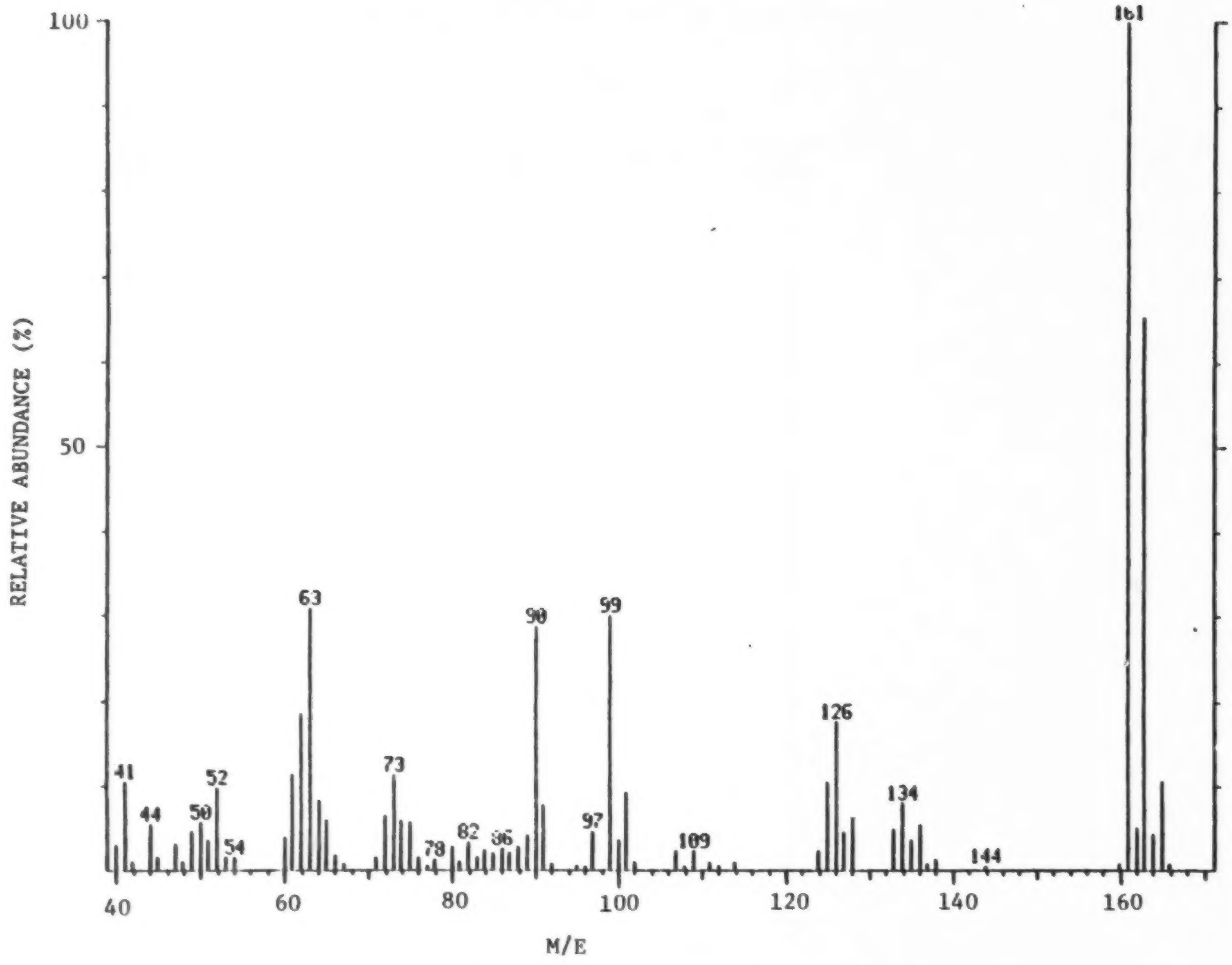

FIGURE D-8. Mass spectrum for 3,4-Dichloroaniline using packed column GC-MS.

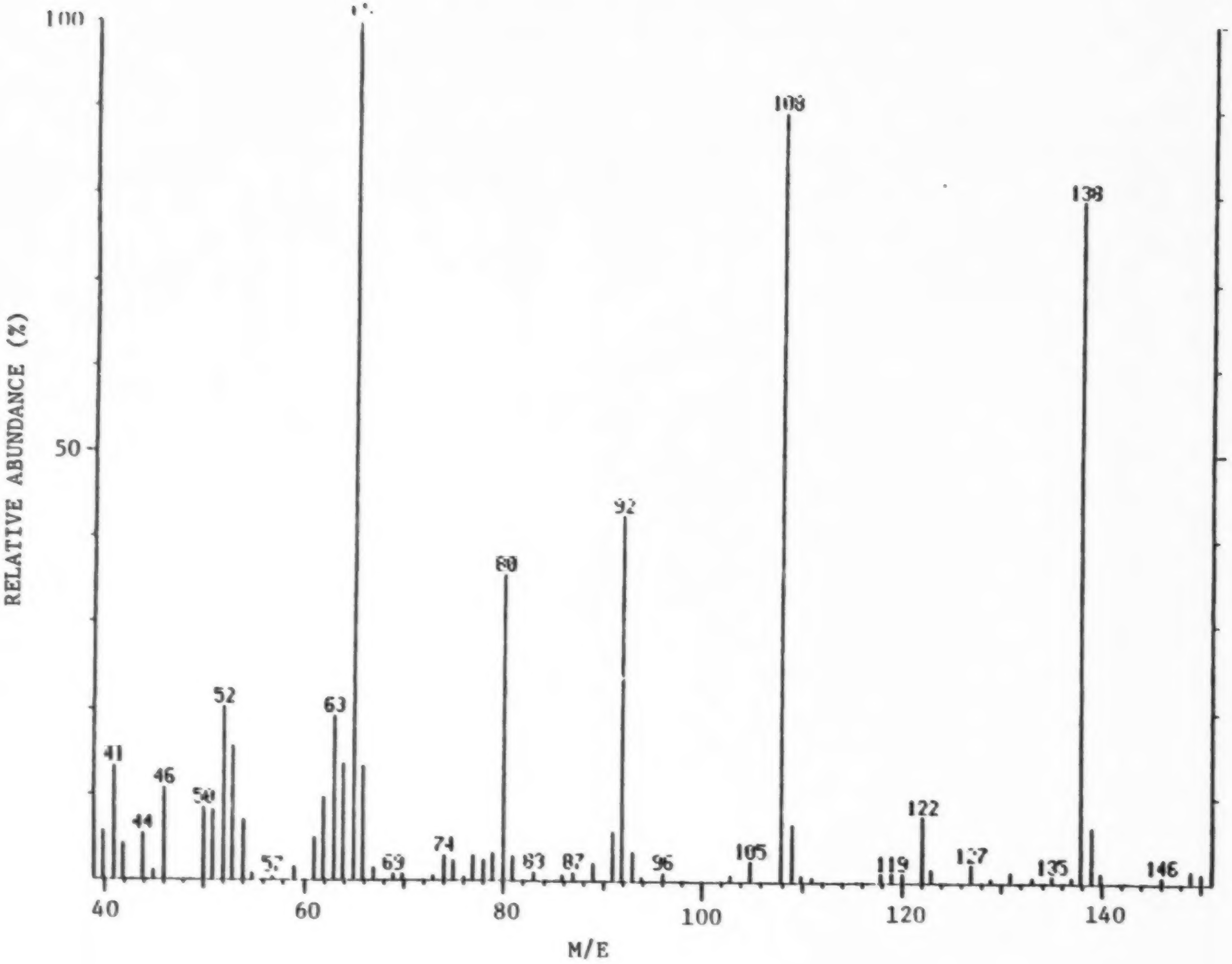

FIGURE D-9. Mass spectrum for 4-Nitroaniline using packed column GC-MS.

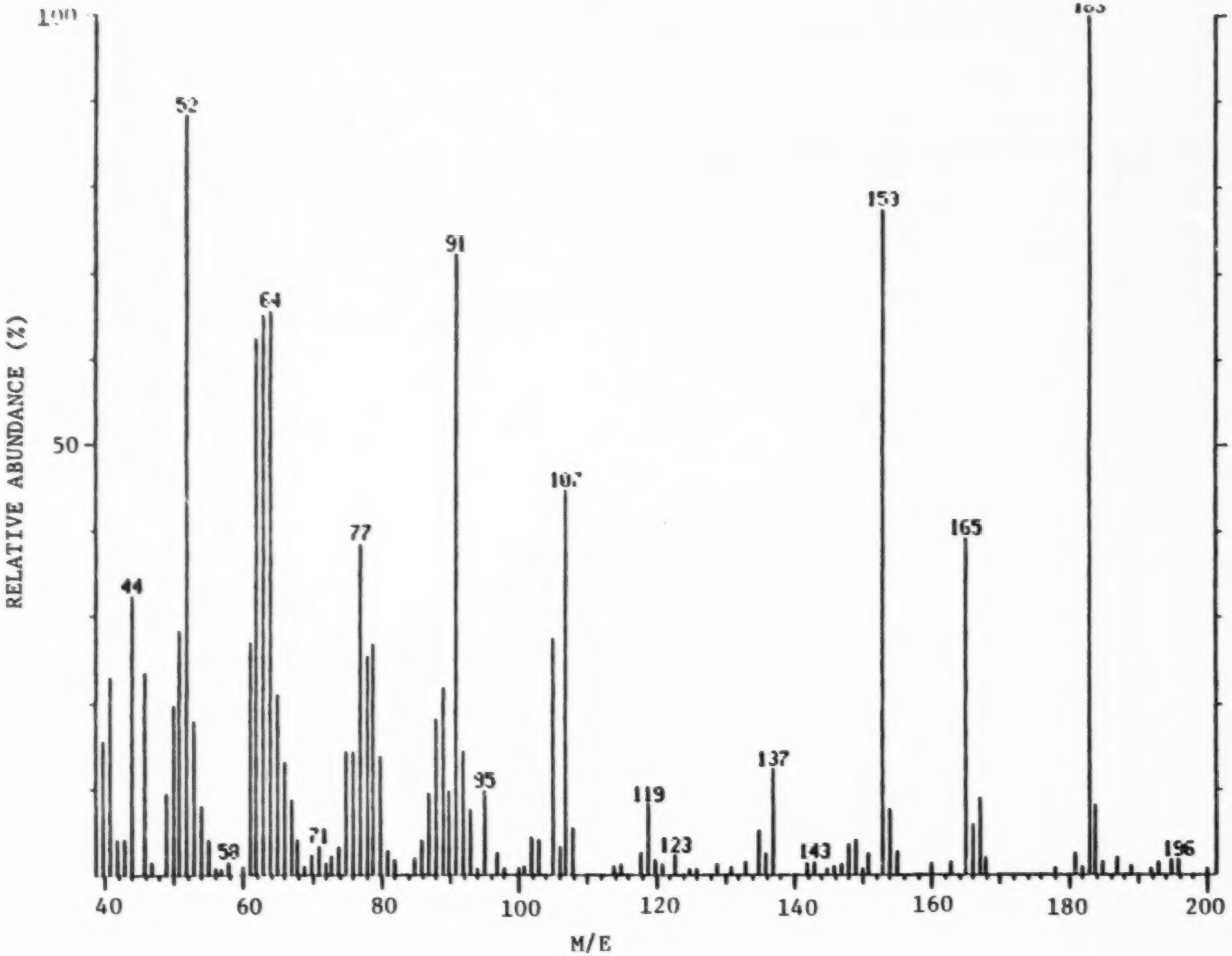

FIGURE D-10. Mass spectrum for 2,4-Dinitroaniline using packed column GC-MS.

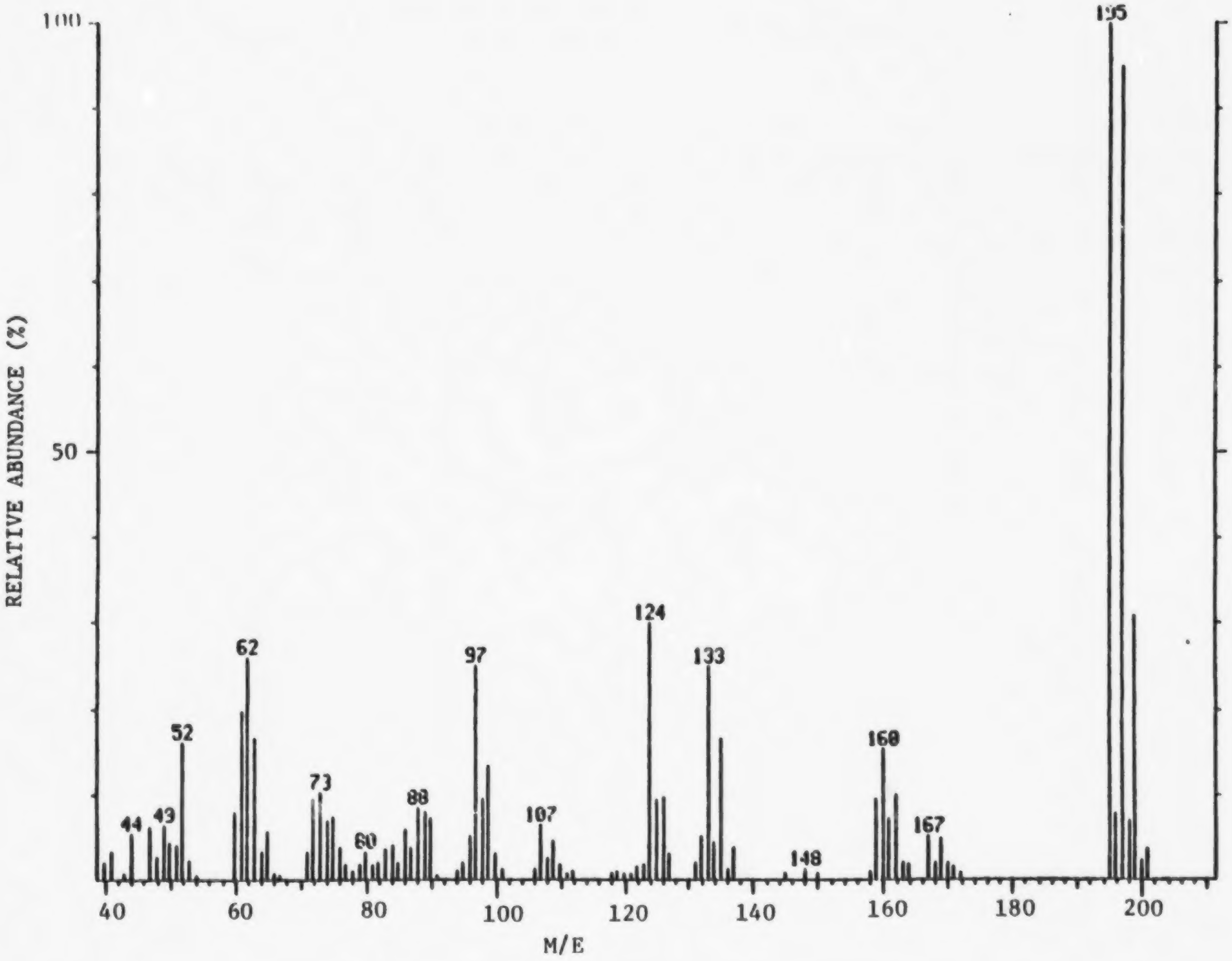

FIGURE D-11. Mass spectrum for 2,4,5-Trichloroaniline using packed column GC-MS.

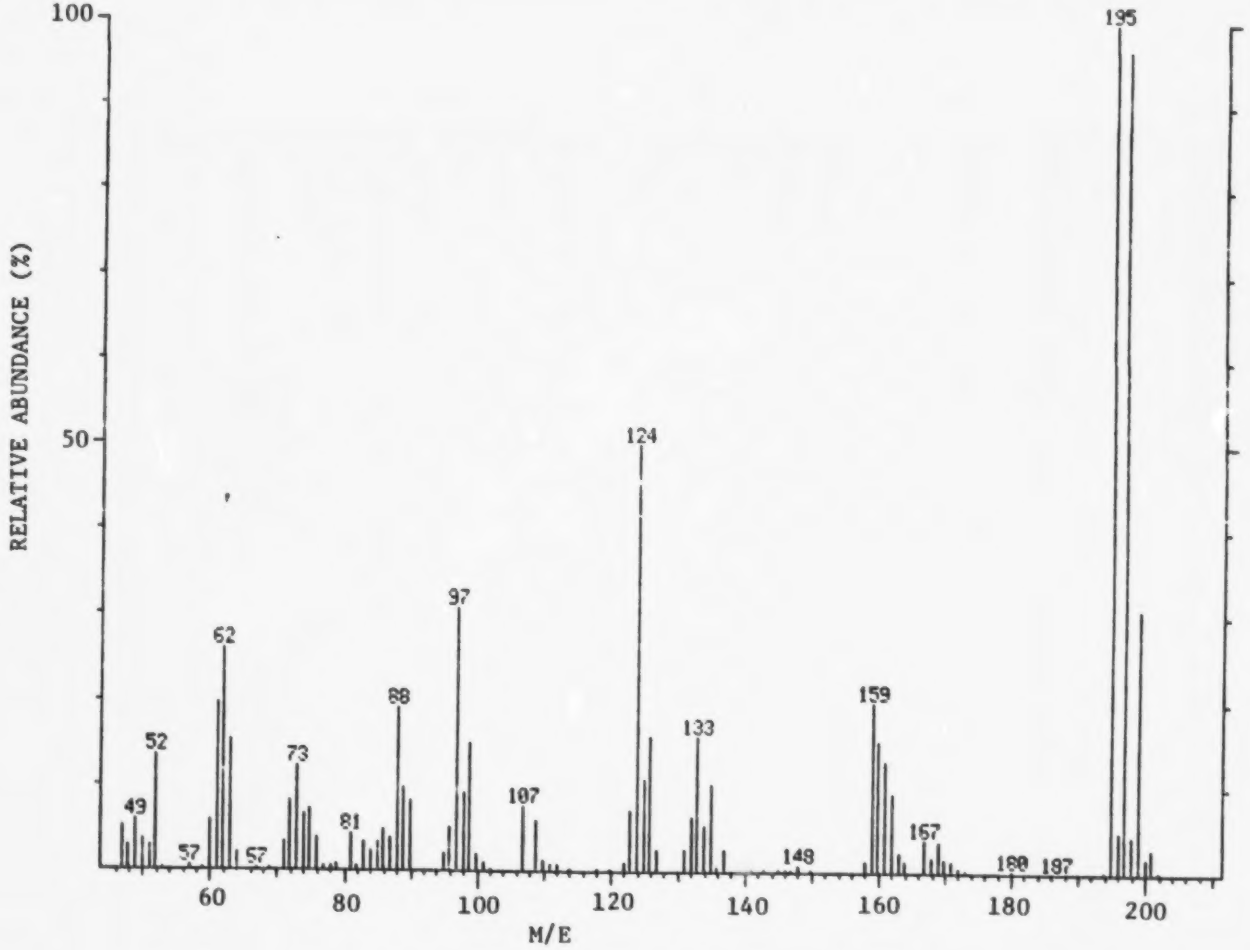

FIGURE D-12. Mass spectrum for 2,4,6-Trichloroaniline using packed column GC-MS.

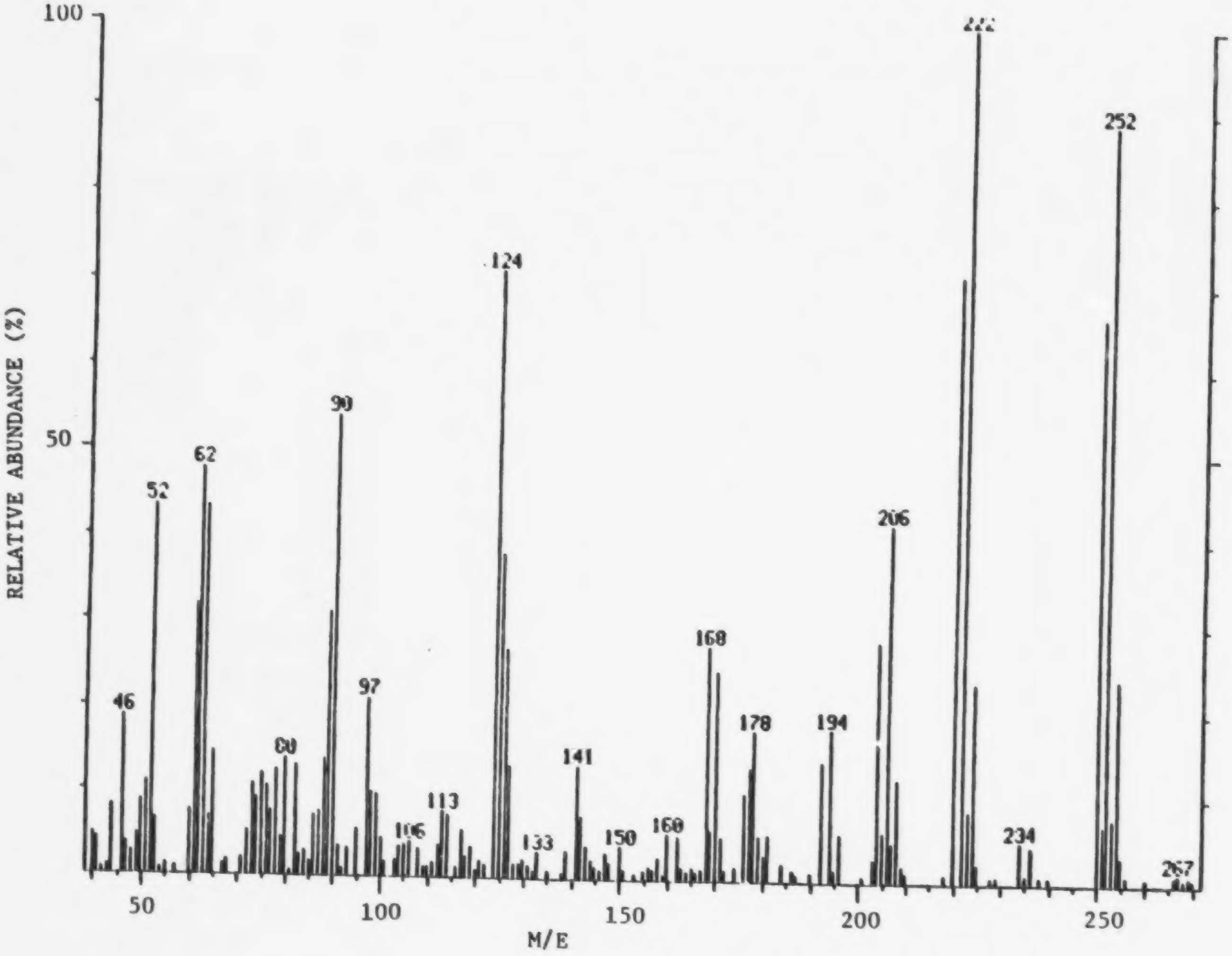

FIGURE D-13. Mass spectrum for 2-Bromo-6-chloro-4-nitroaniline using packed column GC-MS.

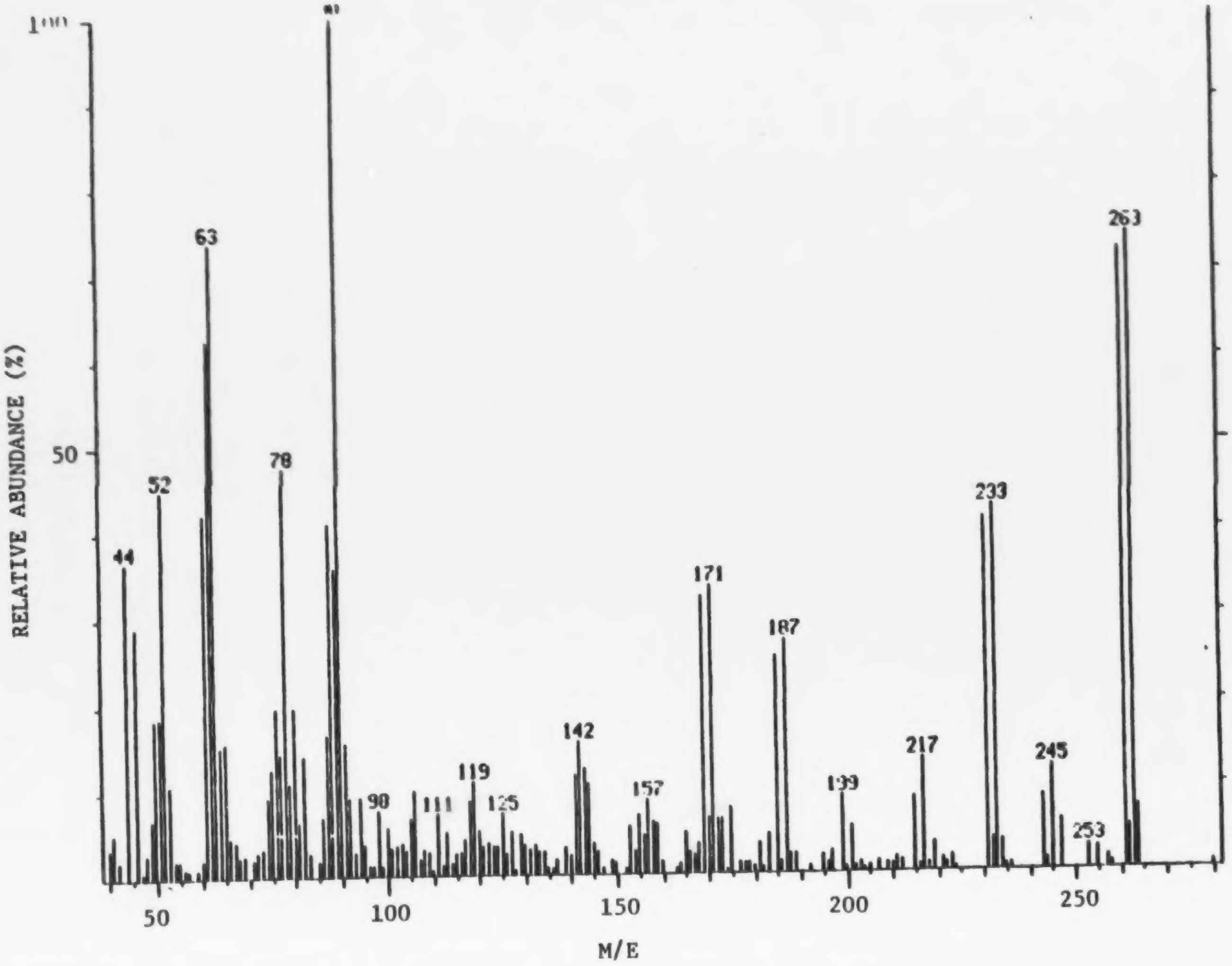

FIGURE D-14. Mass spectrum for 2-Bromo-4,6-dinitroaniline using packed column GC-MS.

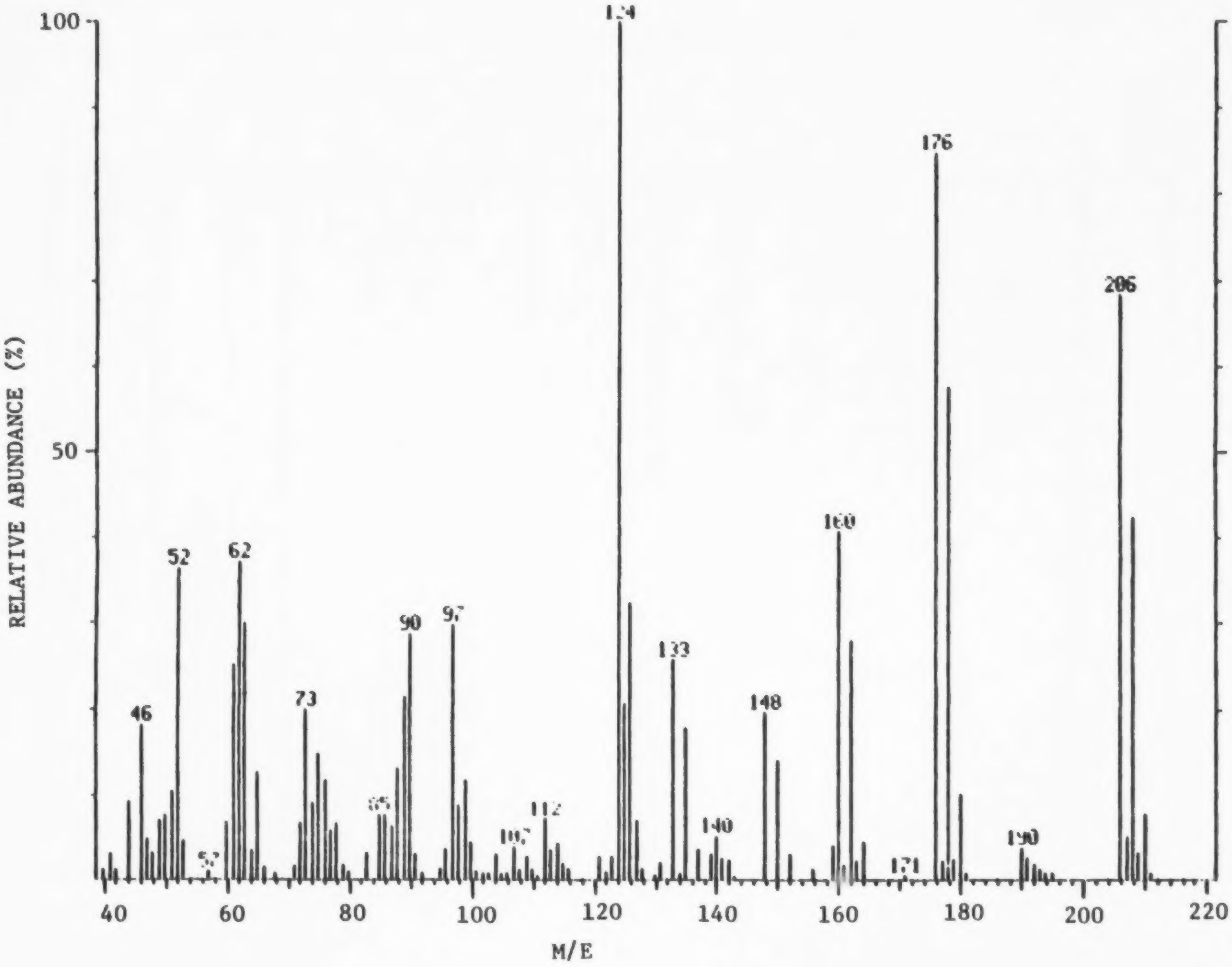

FIGURE D-15. Mass spectrum for 2,6-Dichloro-4-nitroaniline using packed column GC-MS.

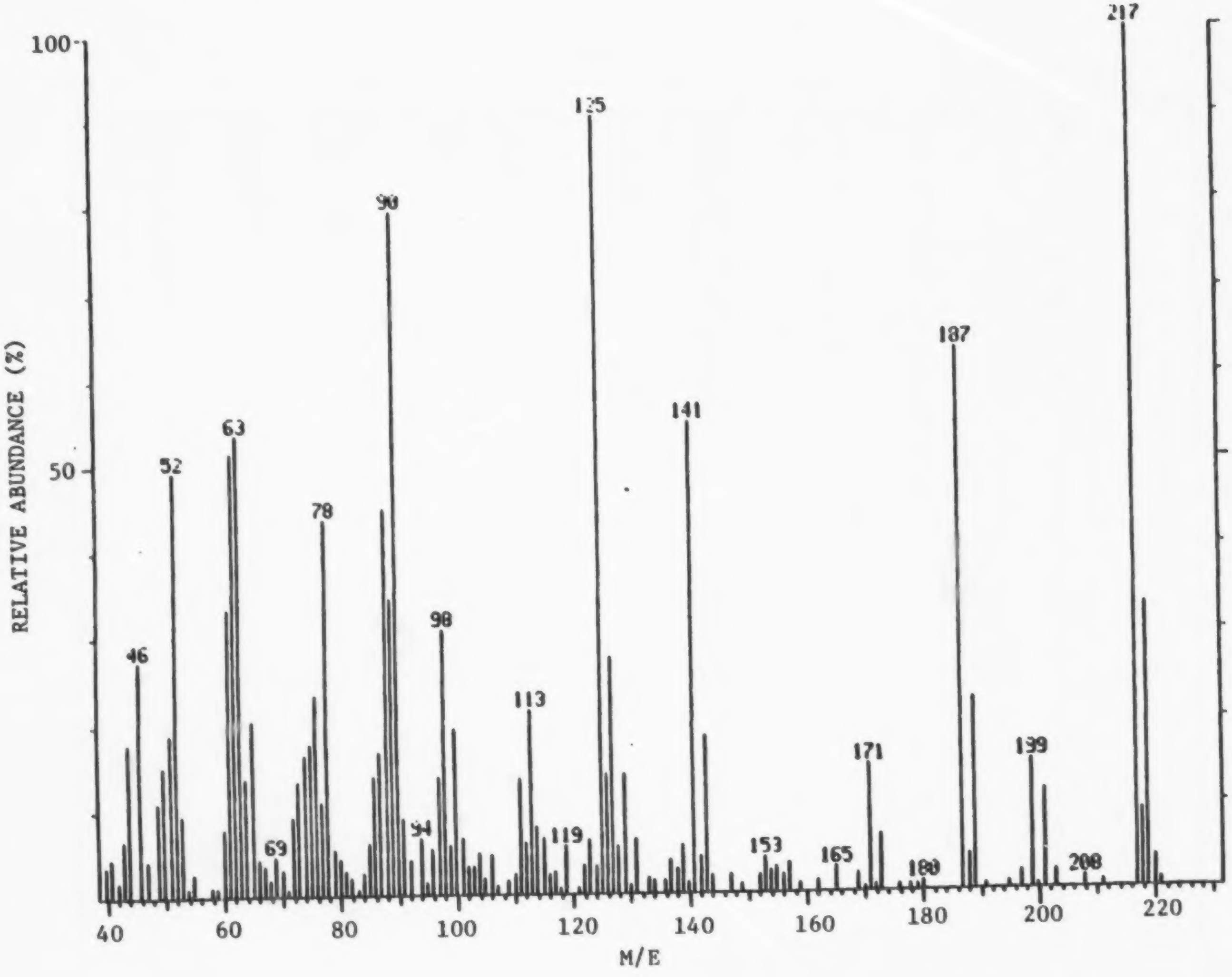

FIGURE D-16. Mass spectrum for 2-Chloro-4,6-dinitroaniline using packed column GC-MS.

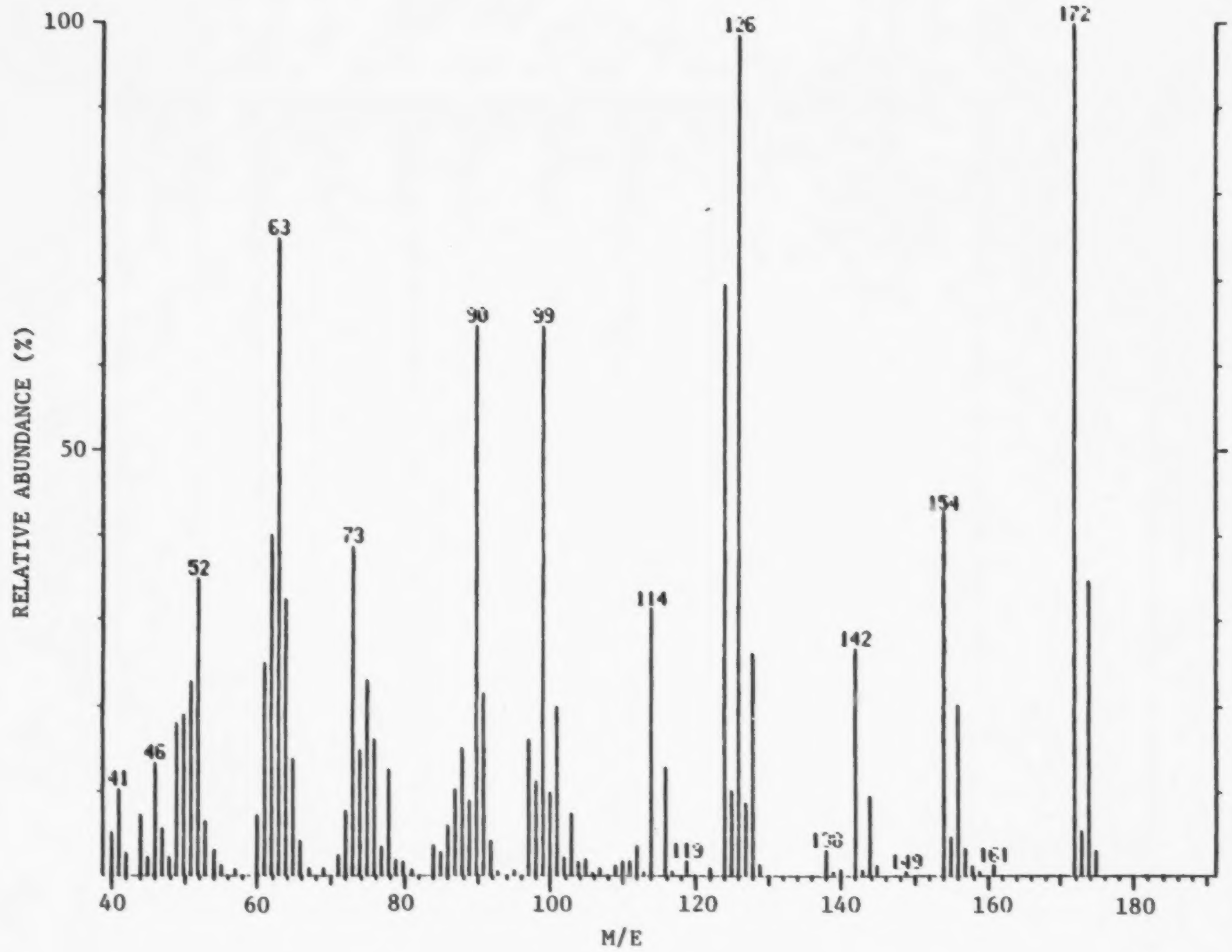

FIGURE D-17. Mass spectrum for 4-Chloro-2-nitroaniline using packed column GC-MS.

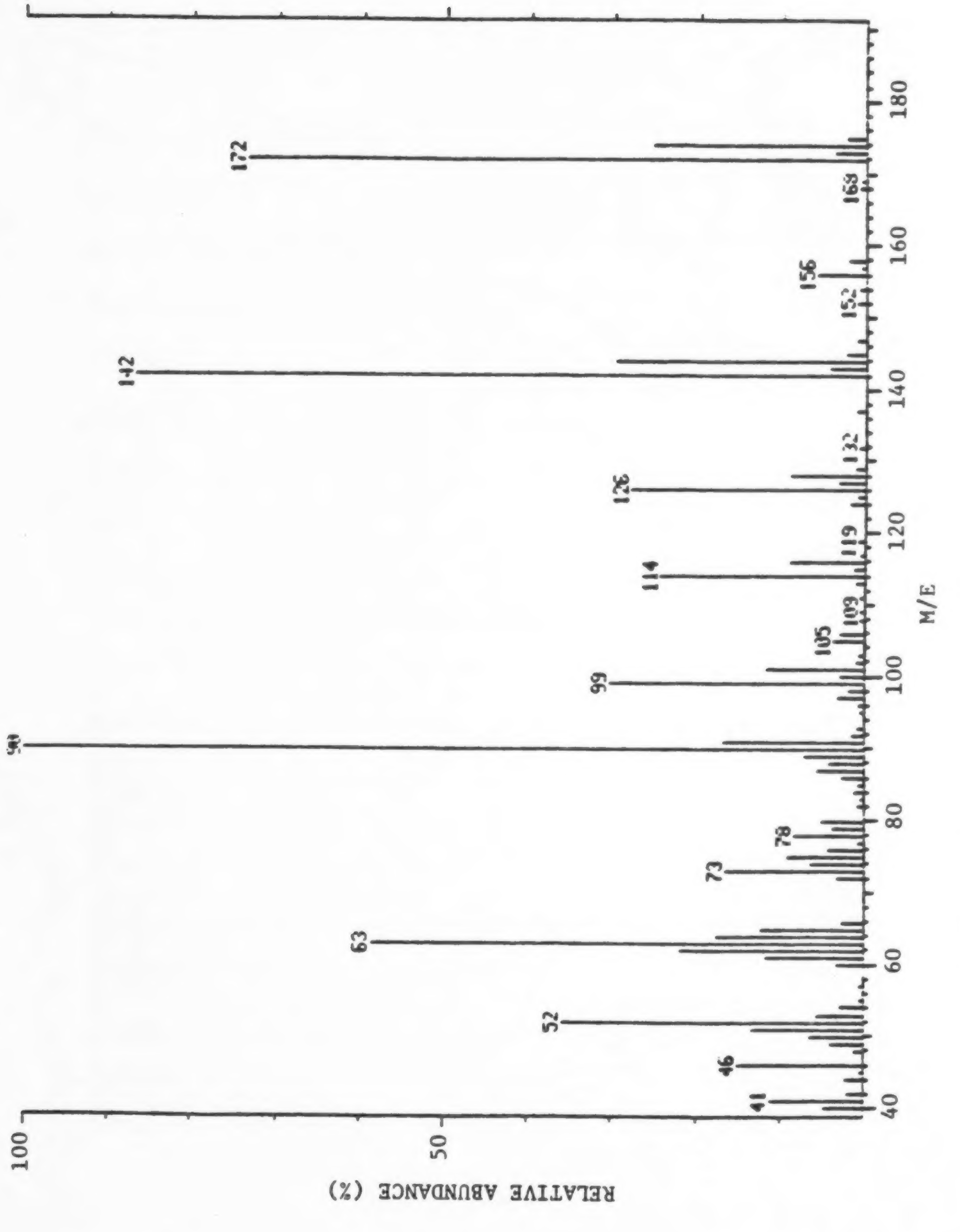

FIGURE D-18. Mass spectrum for 2-Chloro-4-nitroaniline using packed column GC-MS.

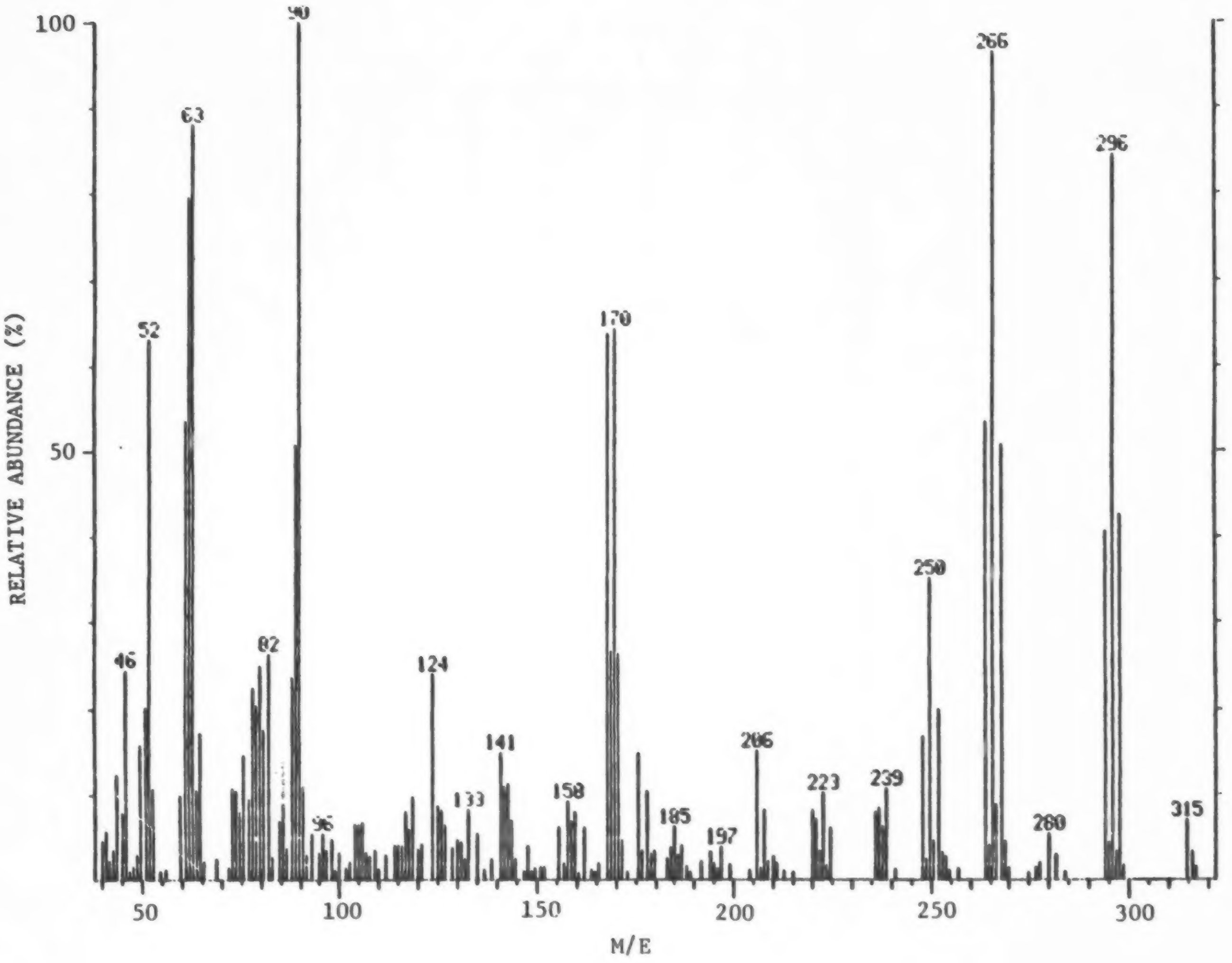

FIGURE D-19. Mass spectrum for 2,6-Dibromo-4-nitroaniline using packed column GC-MS.

APPENDIX E

METHOD 625-S

PROTOCOL FOR THE ANALYSIS OF EXTRACTABLE ORGANIC PRIORITY POLLUTANTS IN INDUSTRIAL AND MUNICIPAL WASTEWATER TREATMENT SLUDGE

1. Scope and Application

1.1 This method is used for the determination of the base, neutral, and acid-extractable organic compounds listed in Table 1.

1.2 The method is for qualitative and quantitative analysis of these compounds in municipal and industrial wastewater treatment sludges. The procedure requires the use of a gas chromatograph/mass spectrometer (GC/MS) as the final detector.

1.3 The method detection limit for each compound is dependent on the compound characteristics and the particular sludge analyzed. However, typical detection limits are 200 to 300 μg/liter for publicly owned treatment works (POTW) sludges with 1 to 5% total solids.

1.4 This method is restricted to use by or under the close supervision of experienced analysts. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.

2. Summary of Method

This method (Figure 1) uses repetitive solvent extraction aided by a high-speed homogenizer. The extract is separated by centrifugation and removed with a pipette or syringe. Sludges are extracted at pH ≥ 11 to isolate base/neutral compounds and at pH ≤ 2 to isolate acidic compounds. Extracts containing base/neutral compounds are cleaned by silica gel or florisil chromatography or by gel permeation chromatography (GPC). Extracts containing the acidic compounds are cleaned by GPC. The organic priority pollutants are determined in the cleaned extracts by capillary column or packed column GC/MS. Option A, i.e., extract cleanup by silica gel or florisil chromatography and analysis by capillary GC/MS (HRGC/MS) is preferred since HRGC/MS allows easier data interpretation. Qualitative identification of compounds is performed using the retention time and the relative abundance of three characteristic ions. Quantitative analysis is performed using external or internal standard techniques with a single characteristic ion.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in GC/MS chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.4.

3.1.1 Glassware must be scrupulously cleaned. All glassware should be cleaned as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and reagent water. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 min. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality dichloromethane may be substituted for the muffle furnace heating. Volumetric glassware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. It should be stored inverted or capped with aluminum foil. .

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the wastewater treatment system being sampled. The cleanup procedures in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve acceptable detection limits.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. However, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis.

4.2 The following parameters covered by this method have been tentatively classified as known or suspected, human or mamalian carcinogens; benzo[a]anthracene, benzidine, 3,3'-dichlorobenzidine, benzo[a]pyrene, α-BHC, β-BHC, δ-BHC, γ-BHC, dibenzo[a,h]anthracene, N-nitrosodimethylamine, 4,4'-DDT, and polychlorinated biphenyls.

5. Apparatus and Materials

5.1 Sampling, Extraction, and Extract Cleanup

5.1.1 Emulsifier-Tekmar Tissumizer® or equivalent, high capacity.

5.1.2 Centrifuge, capable of handling 200 ml bottles.

5.1.3 Centrifuge bottles with TFE lined screw caps, 200 ml.

5.1.4 Kuderna-Danish (K-D) glassware.

5.1.4.1 Snyder columns, 3 bulb, macro.

5.1.4.2 Snyder columns modified micro.

5.1.4.3 Evaporating flasks, 500 ml, 250 ml.

5.1.4.4 Receiver ampuls 5 and 10 ml, graduated, with spring attachment.

5.1.4.5 Beakers, 100 ml.

5.1.5 Water or steam bath for Kuderna-Danish concentrations.

5.1.6 Chromatographic (drying) Column - Pyrex (400 mm x 20 mm ID) without a fritted plate.

5.1.7 Separatory funnels, 500 ml with TFE stopcock.

5.1.8 Syringe, 100 ml, Pyrex, with long needle.

5.1.9 Graduated cylinder, 100 or 250 ml.

5.1.10 Vials, 2 ml, 4 ml, and 8 ml with TFE lined screw caps.

5.1.11 Sample bottles, 1,000 ml or 4,000 ml glass with TFE lined screw caps.

5.1.12 Disposable pipettes, for transferring extracts.

5.1.13 Gel Permeation Chromatograph (GPC), Analytical Biochemical Labs, Inc., GPC Autoprep 1002 or equivalent including:

5.1.13.1 Glass column 25 mm ID x 60-70 mm packed with 70 g of Bio-Beads SX-3.

5.1.13.2 Chromatographic pump, operated at 5 ml/min with 350-700 millibars (5-10 psi).

5.1.13.3 Injector with loop.

5.1.13.4 Syringe filter holder, stainless steel and TFE, Gelman 4310 or equivalent.

5.1.13.5 Spectrophotometric chromatographic detector, 254 nm, with strip chart recorder (optional, for GPC calibration).

5.1.14 Chromatographic column, 200 mm x 20 mm ID, with solvent reservoir (250 ml) and TFE stopcock.

5.1.15 Roller mill.

5.1.16 Bottles, 500 ml, brown glass.

5.2 For Identification and Quantitation

5.2.1 Gas chromatograph/mass spectrometer with data system, Finnigan 4000 or equivalent. The GC/MS interface should include a glass jet separator and a direct capillary line. The computer system should allow acquisition and storage of repetitive scan data throughout the GC/MS runs. Computer software should be available to allow searching of GC/MS data for display of extracted ion current profiles (EICPs) and integration of the peaks. The GC injection port must be designed for on-column injection when using packed columns and for splitless or on-column injection when using capillary columns. GC columns required are:

5.2.1.1 0.9 to 1.8 m x 2 mm ID glass packed with 1% SP-1240-DA on 100/120 mesh Supelcoport or equivalent.

5.2.1.2 15-30 m x 0.2 mm ID wall coated open tubular capillary column coated with SE-54, or equivalent, providing at least 25,000 effective theoretical plates, measured at C_{13}, or a 1.8 m x 2 mm ID glass packed with 3% SP-2250 on 100/120 mesh Supelcoport, or equivalent.

5.2.2 Gas chromatograph/flame ionization detector with the same GC columns as for the GC/MS system.

6. Reagents

6.1 For Extraction and Extract Cleanup

6.1.1 Dichloromethane, "Distilled in Glass" or equivalent, stored in original containers and used as received.

6.1.2 Hexane, "Distilled in Glass" or equivalent, stored in original containers and used as received.

6.1.3 Acetone, "Distilled in Glass" or equivalent, stored in original containers and used as received.

6.1.4 Hydrochloric acid solution (6N). Slowly add 100 ml of HCl (12N) to 100 ml of reagent water.

6.1.5 Sodium hydroxide solution (6N). Dissolve 24 g NaOH in reagent water and dilute to 100 ml.

6.1.6 Sodium sulfate (Na_2SO_4), Anhydrous, granular. Clean by overnight Soxhlet extraction with dichloromethane, drying in an oven at 110 to 160°C oven, and then heating to 650°C for 2 hr. Store in a glass jar tightly sealed with TFE-lined screw cap.

6.1.7 Silica gel, 75/150 mesh. Clean silica gel for 16 h by Soxhlet extraction with dichloromethane. Dry and activate for 16 h at 160°C. Deactivate by adding 3% water (by weight) and mixing for at least 4 h on a tumbler. Store at room temperature in glass jars fitted with TFE lined screw caps.

6.1.8 Florisil 60/100 mesh, Floridan. Clean florisil for 16 hr by Soxhlet extraction with dichloromethane. Dry and activate for 16 h at 160°C. Deactivate by adding 1% water by weight and mixing for at least 4 h on a tumbler. Store at room temperature in glass jars fitted with TFE lined screw caps.

6.1.9 Glass wool. Clean glass wool by thorough rinsing with hexane, dried in a 100°C oven, and stored in a hexane rinsed glass jar with TFE lined screw cap.

6.1.10 Boiling chips - silica or carborundum.

6.1.11 GPC Calibration Solutions

6.1.11.1 Corn oil, 200 mg/ml in dichloromethane.

6.1.11.2 Pentachlorophenol and di-n-octylphthalate, 4 mg/ml each in dichloromethane.

6.2 For Identification and Quantitation

6.2.1 Analytical Standards

6.2.1.1 Prepare stock solutions from the pure compounds by dissolving 10 mg quantities into 10 ml of dichloromethane. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.2.1.2 Transfer the stock standard solutions into TFE sealed screw cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards are available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, that can be used to determine the accuracy of calibration standards.

6.2.1.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicate a problem.

6.2.1.4 Prepare mixed analytical standards by diluting aliquots of the stock solutions. The acids standard should contain each of the phenolic compounds at concentrations in the range of 50 to 200 ng/µl. The base, neutral, and pesticide (B/N/P) standard should be prepared at concentrations in the range of 20-100 ng/µl. All working standards must include d_{10}-anthracene at 20 ng/µl.

6.2.2 Analyte Spiking Solutions

Prepare mixed spiking solutions by serial dilution from the individual stock solutions prepared as described in Section 6.2.1. The specifications for spiking solutions for individual samples are described in Section 8.3.2.

6.2.3 Internal Standard Spiking Solution

Prepare d_{10}-anthracene internal spiking solutions at 20 µg/liter in dichloromethane as described in Section 6.2

6.2.4 Surrogate Standard Spiking Solution

Select a minimum of five B/N and two acid surrogate compounds from Table 2. Prepare and store stock standard and spiking solutions as described in Section 6.2.1.

6.2.5 DFTPP Standard

Prepare a 25 ng/µl solution of DFTPP in dichloromethane.

7. Method Calibration

7.1 Establish GC/MS operating parameters equivalent to those indicated in Section 12. The GC/MS system can be calibrated using the external standard technique or the internal standard technique.

7.2 External Standard Calibration Procedure

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with dichloromethane. One of the external standards should be at a concentration near, but above, the method detection limit and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 1 to 2 µl of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected in the calibration factor is a constant over the working range (< 10% relative standard deviation RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3 Verify the working calibration curve or calibration factor on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ± 10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal Standard Calibration Procedure. To use this approach, select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. The internal standards used for this procedure must include d_{10}-anthracene.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards and dilute to volume with dichloromethane. One of the standards should be at a concentration near, but above, the method detection limit, and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Using injections of 2 to 5 µl of each calibration standard, tabulate peak height or area responses against concentration for each compound and internal standard, and calculate relative response factors (RRF) for each compound using Equation 1.

$$RRF = (A_s B_{is})/(A_{is} B_s) \qquad \text{(Eq. 1)}$$

where A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

B_{is} = Mass of the internal standard (ng).

B_s = Mass of the parameter to be measured (ng).

If the RRF value over the working range is a constant (< 10% RSD), the RRF can be assumed to be nonvariant and the average RRF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is} vs. RRF.

7.3.3 Verify the working calibration curve or RRF on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ± 10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Daily Calibration of the GC/MS System

7.4.1 At the beginning of each day, check the calibration of the GC/MS system and adjust if necessary to meet decafluorotriphenylphosphine (DFTPP) specifications (Section 7.4.3). Each day base/neutrals are measured, the column performance specification (Section 12) with benzidine must be met. Each day the acids are measured, the column performance specification (Section 12) with pentachlorophenol must be met. DFTPP can be mixed in solution with either of these compounds to complete two specifications with one injection, if desired.

7.4.2 To perform the calibration test of the GC/MS system, the following instrumental parameters are required.

Electron energy, 70 eV (nominal)

Mass range, m/e 40-475

Scan time to provide at least 5 scans per peak. Typical scan times are 3 s or less for acids or base/neutrals with the packed column, 1 s or less for base/neutrals with the capillary column.

7.4.3 Evaluate the system performance each day that it is to be used for the analysis of samples or blanks by examining the mass spectrum of DFTPP. Inject a solution containing 50 ng DFTPP and check to ensure that performance criteria listed in Table 3 are met. If the system performance criteria are not met, the analyst must retune the spectrometer and repeat the performance check. The performance criteria must be met before any samples or standards may be analyzed.

7.4.4 At the beginning of each working day, verify the calibration of the GC/MS system by injecting a standard mixture (Section 7.3.3).

7.4.5 For instrument performance verification, to each acid and base/neutral extract and each standard analyzed, add an amount of d_{10}-anthracene immediately prior to injection such that 40 ± 4 ng will be injected.

8. Quality Control

8.1 Demonstrate Acceptable Performance

Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this procedure.

8.1.1 For each compound to be measured, select a spike concentration representative of the expected levels in the samples. Using stock standards, prepare a quality control check standard in acetone 1,000 times more concentrated than the selected concentrations.

8.1.2 Add 80 µl of the check standard to each of a minimum of four 80 ml aliquots of reagent water with a syringe. A representative sludge can be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.1.3 Calculate average recovery (R) and standard deviation (s), in percentage recovery, for the results. Sludge background corrections must be made before R and s calculations are performed.

8.1.4 Using the appropriate data from Tables 12-14, determine the recovery and single operator precision expected for the method for each parameter, and compare these results to the values calculated in Section 8.1.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.

8.2 Precision and Accuracy Statement. The analyst must calculate method performance criteria for each of the surrogate standards.

8.2.1 Calculate upper and lower control limits for method performane for each surrogate standard, using the values for R and s calculated in Section 8.1.3:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R - 3 s

The UCL and LCL can be used to construct control charts[4] that are useful in observing trends in performance.

8.2.2 For each surrogate standard, the laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of sludge as described in Section 8.1.2, followed by the calculation of R and s. Alternately, the analyst must use four sludge data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[4]

8.3 Surrogate Spikes. The laboratory is required to spike all of their samples with the surrogate standard spiking solution to monitor spike recoveries. Suggested surrogate compounds are listed in Table 5. If the recovery for any surrogate standard does not fall within the control limits for method performance, the results reported for that sample must be qualified as described in Section 14.3. The laboratory should monitor the frequency of suspect data to ensure that it remains at or below 5%.

8.4 Method Blank. Before processing any samples, the analyst should demonstrate through the analysis of an 80 ml aliquot of reagent water, that all glassware and reagents are interference free. Each time a set of samples is extracted or there is a change in reagents, a laboratery reagent blank should be processed as a safeguard against laboratory contamination.

8.5 Fortified and Replicate Samples

8.5.1 Sample Selection. Select at least 30% of all samples or at least one sample from each sampling location for fortified and replicate aliquot analysis. Analyze the selected samples in duplicate to determine unspiked analyte concentrations. Using the procedures described in Section 8.5.2, spike and analyze duplicate aliquots of the selected samples. Analyze a third unspiked aliquot of the selected samples on the same day that the corresponding spiked aliquots are analyzed.

8.5.2 Spiking Procedures. Spike an 80 ml aliquot of sludge with the representative semivolatile compounds listed in Table 4 and with other compounds identified in the sample, if available, at two times the observed concentration or at 10 times the lower limit of detection, whichever is greater. Prepare the spike as two acetone solutions with the acidic and neutral compounds in the first solution and the basic compounds in the second solution. The concentrations of the spiking solutions should be such that 1 to 5 ml of each solution are added to the sludge sample to attain the required spike concentration. Homogenize the spiked sample for 45 to 60 s and store overnight at 4°C with tumbling before extraction and analysis.

9. Sampling and Preservation

9.1 Sampling. Collect samples in glass containers (1,000 to 4,000 ml) with a TFE lined screw cap. The container should be prewashed with acetone and dried before use. Containers should be filled no more than two-thirds full with sample to minimize breakage during freezing.

9.2 Preservation. Preferably, samples should be iced or refrigerated at 4°C for not more than 24 h before extraction. Where extraction cannot be performed within 24 h, samples should be frozen. Samples may be stored for up to 30 days at -20°C or indefinitely at -75°C. In order to prevent breakage during storage, the containers should not be slightly warmed and then recooled.

10. Sample Extraction

10.1 Preparation of Drying Column. Immediately prior to extracting a sample, prepare a drying tube for the extract. Place a small glass wool plug in the bottom of the column and add anhydrous sodium sulfate to a depth of 10 to 15 cm.

10.2 pH Adjustment. Thoroughly homogenize the sludge sample by mixing with an emulsifier in the sample bottle for 1 min, then quickly remove a 80 ml aliquot into a 250 ml graduated cylinder. Transfer the aliquot into a 250 ml centrifuge bottle. Basify to pH ≥ 11 with a 6 N sodium hydroxide solution. Mix briefly with the homogenizer to ensure uniform sample pH. (Note: If copious precipitation of carbonates is observed when sodium hydroxide is added, make the sample slightly acidic with 6 N hydrochloric acid and allow the carbon dioxide evolution to cease before basifying the sample.)

10.3 Basic Extraction. Add 80 ml of dichloromethane to the centrifuge bottle and homogenize for 45 to 60 s. Do not homogenize more than 60 s to avoid heating the sample. Cap the centrifuge bottles with TFE-lined screw caps and centrifuge at 2,500 to 3,000 rpm for 30 min. Stir and repeat centrifugation if satisfactory phase separation is not achieved. The mixture will separate into an aqueous layer over the dichloromethane extract with a solids cake at the water-dichloromethane interface. Withdraw the extract from each centrifuge bottle with a 100 ml syringe. Discharge the extracts into a 500 ml separatory funnel. Drain the dichloromethane through the drying column into a Kuderna-Danish evaporator. Retain any aqueous layer and return it to the centrifuge bottle. Extract the sample two more times to achieve three-fold extraction. Wash the drying column with an additional 100 ml of dichloromethane and combine the eluent with the dried extracts.

10.4 Extract Concentration. Add a boiling chip to the extract in the Kuderna-Danish evaporator and concentrate the extract to 8 ml using an 85°C bath or a steam bath. If the extract is only slightly colored and not notably viscous, fit a modified Snyder column onto the Kuderna-Danish receiving tube and immerse the tube halfway in a 35°C water bath. Direct a gentle stream of nitrogen directly on the surface of the extract until the volume is ≤ 4 ml. Transfer the extract to a clean vial, seal it with TFE lined screw cap, and store the extract at 4°C. If the extract is highly colored, viscous or solidifies when concentrating to 4 ml, dilute the extract to 12 ml with dichloromethane, transfer to a clean vial, and store as described above.

10.5 Acidic Extraction. Acidify the sludge sample portions to pH ≤ 2 with 6 N hydrochloric acid and extract the sample again by procedures described in Sections 10.3 to 10.4. Discard the extracted sludge aliquots.

11. Extract Cleanup

11.1 Base/Neutral Extracts. Clean the base/neutral sludge extracts by adsorption chromatography on either florisil or silica gel. Although there does not appear to be any significant difference in the performance of these adsorbents, the adsorbent selected for analyses of all quality assurance blanks, spiked blanks, and spiked sludges associated with a specific sludge sample must be the same as was selected for the original sample analysis. In cases where the base/neutral extracts are to be analyzed by packed column GC/MS, the GPC procedure (Section 11.2) may be used as an alternative cleanup method.

11.1.1 Column Preparation. Prepare a 200 mm x 20 mm ID silica gel or florisil column by pouring 20 g fresh 3% deactivated silica gel or fresh 1% deactivated florisil, into a chromatographic column containing 60 to 70 ml hexane. Tap the side of the clamped column with a stirring rod to aid packing and air bubble escape. If clumping should occur at the bottom of the solvent reservoir, a stirring rod can be used to break the clumps. Add hexane as needed. After most packing has settled, rinse down the florisil or silica gel adhering to the column walls with additional hexane. Wash the column with an additional 20 ml hexane until the solvent level is within 1 to 2 mm of the top of the silica gel or florisil.

11.1.2 Column Calibration.

11.1.2.1 Check the elution pattern for each new batch of adsorbent prepared by chromatographing a spiked blank. Mix 1 ml of a dichloromethane solution containing 100 μg of each of the specific analytes of interest and the representative B/N/P compounds included in quality assurance method spikes (described in Section 8.3) with 2 g of adsorbant. Evaporate the solvent with a gentle stream of dry nitrogen and add the adsorbant to the column.

11.1.2.2 Elute the column with 20 ml of hexane (Fraction I), 50 ml of 10% dichloromethane in hexane (Fraction II), 50 ml of 50% dichloromethane in hexane (Fraction III), and then 150 ml of 5% acetone in dichloromethane (Fraction IV). Maintain the elution flow at 2 to 4 ml/min.

11.1.2.3 Collect each fraction in separate Kuderna-Danish evaporators and concentrate the fractions according to the procedures described in Section 7.4.

11.1.2.4 Analyze each fraction by GC/FID or GC/MS with the SE-54 capillary or packed SP-2250 column. Determine the recovery of each compound in each fraction by comparing the detector responses with those from a duplicate aliquot of the original spiked blank. Elution patterns and recoveries observed for selected compounds chromatographed on silica gel and florisil are shown in Tables 5 and 6.

11.1.2.5 Increasing the volume of Fraction I may be required to provide acceptable cleanup for sludge extracts containing high concentrations of aliphatic hydrocarbons or other non-polar compounds. However, additional hexane may elute significant portions of the chlorinated benzenes, hexachloroethane, hexachlorobutadiene, hexachlorocyclopentadiene, 2-chloronaphthalene, aldrin, and p,p'-DDE. The elution patterns and recoveries observed for many B/N/P compounds chromatographed on silica gel using 100 ml of hexane for Fraction I are shown in Table 7. In cases where the volume of Fraction I must be increased to achieve acceptable cleanup and the analytes of interest include the compounds listed in this section, Fraction I must be analyzed separately from Fraction II-IV.

11.1.3 Column Operation.

11.1.3.1 Place 2 g of fresh 1% deactivated florisil or 3% deactivated silica gel into a small beaker and add the concentrated base/neutral extract. Dry the sample with a gentle nitrogen stream with stirring to ensure fast and uniform drying. Load dried florisil or silica gel containing the sludge components into the column. Elute the column as described in Section 11.1.2. Discard Fraction I.

11.1.3.2 Collect and composite in Kuderna-Danish evaporators all fractions containing the compounds of interest such as Fractions II through IV for all B/N/P compounds. Fraction II frequently contains much higher levels of interfering materials than to Fractions III or IV. As a result, it should not be composited with Fractions III and IV if it contains no compounds of interest. Fractions may be analyzed separately if desired.

11.1.3.3 Concentrate all fractions collected to 1 ml according to procedures described in Section 10.4.

11.2 Acidic Fraction. Gel permeation chromatography is used to remove triglycerides and fatty acids.

11.2.1 GPC Column Preparation. Place 70 g of Bio-Beads SX-3 in a 400 ml beaker. Cover the beads with dichloromethane and allow the beads to swell overnight before packing the columns. Transfer the swelled beads to the column and start pumping solvent, dichloromethane, through the column, with upward flow at 5.0 ml/min. After ~ 1 h, adjust the pressure on the column to 350 to 700 millibars (5 to 10 psi) and pump an additional 4 h to remove air from the column. Adjust the column pressure periodically as required to maintain 350 to 700 millibars.

11.2.2 Column Calibration.

11.2.2.1 Calibrate the GPC column elution at least once a week according to the following procedure. Load 5 ml of the corn oil solution into sample loop No. 1 and 5 ml of the phthalate-phenol solution into loop No. 2. Inject the corn oil and collect 10 ml fractions, for 36 min, changing the fraction at 2 min intervals. Inject the phenols solution and collect 10-ml fractions for 1 h.

11.2.2.2 Determine the corn oil elution pattern by evaporation of each fraction to dryness followed by a gravimetric determination of the residue.

11.2.2.3 Analyze the phthalate-phenol fractions by GC/FID on the SP-2250 and SP-1240-DA columns. Plot the concentration of each component in each fraction versus total eluent volume (or time) from the injection points.

11.2.2.4 Choose a "dump time" which allows ≥ 85% removal of the corn oil and ≥ 85% recovery of the di-n-octylphthalate. Choose the "collect time" to extend at least 10 min after the elution of pentachlorophenol. "Wash" the column 20 min between samples. Typical parameters selected are: dump time, 20 min (100 ml); collect time, 30 min (150 ml); and wash time, 20 min (100 ml).

11.2.2.5 The column can also be calibrated using a 254-nm UV detector to monitor the elution of the corn oil and the phenols. Measure the peak areas at various elution times to determine appropriate fractions.

11.2.3 Column Operation.

11.2.3.1 Prefilter or load all extracts via the filter holder to avoid particulates that might cause flow stoppage. Load the ≤ 4-ml extracts into the 5-ml loops with a clean solvent on both sides of the extract. Load the ~ 12-ml extracts in three consecutive loops. Use sufficient clean solvent after the extract to transfer the entire aliquot into the loop. Between extracts, purge the sample loading tubing thoroughly with clean solvent. After especially dirty extracts, run a GPC blank using dichloromethane to check for carry-over. Process the extracts using the dump, collect, and wash parameters determined from the calibration and collect the cleaned extracts in 250 ml brown bottles.

11.2.3.2 Concentrate the cleaned extracts, combining collected fractions from multiple injections, to ~ 8 ml using Kuderna-Danish evaporators and then to ≤ 1 ml using modified Snyder columns and nitrogen blowdown.

11.2.3.3 Transfer the cleaned extracts to 8 ml graduated tubes and dilute to 4 ml with dichloromethane. Store at 4°C for GC/MS analysis. Intensely colored extracts may require a second GPC cleanup.

11.2.3.4 Note: Sample extracts must be in the same solvent used for column elution. Injection of solutions in other solvents can cause a significant change in the bead swell. In extreme cases a traumatic pressure increase may damage the column and cap, or even break the glass column.

12. Sample Extract Analysis

12.1 Base/Neutral/Pesticide Extracts. Analyze the B/N/P extracts by GC/MS using the capillary column or packed column systems described in Section 5.2.1 under the conditions shown below. The capillary column GC/MS procedure is preferred because this procedure provides more unambiguous interpretation of the resulting data. However, if satisfactory performance of the column cannot be demonstrated or if the required Grob-type injection system with direct capillary GC/MS interface is not available, the packed column GC/MS procedure may be used.

12.1.1 Capillary GC/MS with a SE-54 WCOT Column

12.1.1.1 Column Temperature: 50°C for 4 min, 50 to 320°C at 4°C/min, and 320°C until after the elution time for benzo[g,h,i]perylene.

GC/MS interface, 300°C

Carrier gas helium at 10 psi

Splitless injection for 30 s

Injection size, 2 µl

Examples of the separations achieved by this column are shown in Figure 2. Relative retention times for the base/neutral compounds on this column are listed in Table 8.

12.1.1.2 Capillary Column GC/MS Performance Checks

12.1.1.2.1 Column Performance Screening. Fully evaluate the performance of each new column by GC/FID prior to installation in the GC/MS system.[5] In addition, recheck columns that show a marked decrease in performance on the GC/MS (as described in Section 12.2.1.3) or that have not been used the previous 2 weeks for GC/MS analyses under this protocol. Install the capillary column in a gas chromatograph equipped with a Grob-type split/splitless injector and flame ionization detector. Adjust carrier gas at about 15 to 20 cm/s for N_2

or 30 to 40 cm/s when He or hydrogen are used as the carrier gas. Set the splitter flow at 30 to 50 ml/min, makeup gas at 30 ml/min, and column oven temperature at 85°C. Using splitless injection, inject 2 µl of a column performance test mixture containing 20 ng each of 2,6-dimethylphenol, 2,6-dimethylaniline, 1-octanol, 2-octanone, *n*-tridecane, and n-tetradecane or an equivalent commercially available test mixture. Table 9 shows the contents of three appropriate commercial mixtures. Measure the pH of the column as the ratio of the peak height of 2,6-dimethylaniline to that of 2,6-dimethylphenol. A value of 0.5 to 1.5 is acceptable. Determine the activity of the column toward polar compounds by determining both the asymmetry for the 1-octanol peak and the ratio of the peak height of 1-octanol to that of C_{13} alkane. The peak asymmetry is evaluated by drawing a perpendicular from the apex of the peak to the baseline and measuring the width from the front of the peak to the perpendicular line (W_F) and from the back of the peak to the perpendicular line (W_B).

$$AS = \frac{W_B}{W_F} \times 100$$

Peak asymmetries between 75 and 200 are acceptable. The ratio of peak height of the 1-octanol to that of C_{13} alkane should be higher than 0.5. Determine the number of effective theoretical plates (N_{eff}) from the tridecane peak using the equation

$$N_{eff} = 5.545 \quad \left(\frac{tr'}{W_{0.5}}\right)^2$$

where tr' is the adjusted retention time of tridecane and $W_{0.5}$ is the peak width at half height. The number of effective plates of acceptable columns must be at least 25,000.

12.1.1.2.2 System Performance. Evaluate the performance of the capillary column in the GC/MS system each time the column is installed and each working day prior to its use. Using splitless injection, inject 2 µl of a test mixture containing 20 ng each of 2,6-dimethylaniline, 2,6-dimethylphenol, methylstearate, octadecene, and octadecane. Measure the pH of the column by taking the ratio of peak heights of 2,6-dimethylaniline to that of 2,6-dimethylphenol. A pH of 0.5 to 1.5 is acceptable. Determine the resolution of the peaks corresponding to octadecane and octadecene. The peaks should be resolved with a 50% valley or better. Determine the asymmetry of the methylstearate peak according to the formula given in Section 12.1.1.2.a. If the methylstearate peak tails, the GC/MS transfer line is not adequately heated.

12.1.2 Packed Column GC/MS with a SP-2250 Column

Analyze B/N extracts by GC/MS using the SP-2250 column described in Section 5.2.1, operated under the following conditions.

Column temperature, 60°C for 2 min, 60 to 260°C at 80°C/min, and 260°C until after the elution time for benzo-[*g*,*h*,*i*]perylene.

Injector temperature, 225°C

GC/MS interface temperature, 275°C

Carrier gas, helium at 30 ml/min

Injection size, 2 µl

An example of the separation achieved by the column is shown in Figure 3. Relative retention times for the base/neutral and pesticide compounds on this column are listed in Table 10.

12.2 Acid Extracts

Analyze acid extracts by GC/MS using the SP-1240-DA column described in Section 5.2.1, operated under the following conditions.

Column temperature, 85°C for 2 min, 85 to 185°C at 10°C/min and 185°C until after the elution time for 4-nitrophenol.

Injector temperature, 185°C.

GC/MS surface temperature, 275°C.

Carrier gas, helium at 30 ml/min.

Injection size, 2 µl.

An example of the separation achieved by the column is shown in Figure 4. Relative retention times for the acid compounds are listed in Table 11.

13. Qualitative and Quantitative Determination

13.1 Using the characteristic mass spectral ions listed in Tables 8 or 10 for the base/neutral and pesticide compounds and in Table 11 for the acid compounds, plot at least three extracted ion current plots (EICPs) for each analyte.

13.2 Identify the presence of analytes by the coincidence of peaks in the characteristic EICPs at the appropriate retention times and with intensities in the characteristic ratios (Tables 8 or 10 and 11).

13.3 Record the area (intensity) of the peak in the EICP for the most intense ion for each compound identified.

14. Calculations

14.1 Determine the concentration of individual compounds in the sample.

14.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from Equation 2:

$$\text{Concentration, } \mu\text{g/liter} = \frac{(M)(V_e)}{(V_i)(V_s)} \qquad \text{(Eq. 2)}$$

where: M = Mass of material injected (ng).

V_i = Volume of extract injected (µl).

V_e = Volume of total extract (ml).

V_s = Volume of wet sludge extracted (liter)

14.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using Equation 3.

$$\text{Concentration, } \mu\text{g/liter} = \frac{(A)(I_s)(V_e)}{(A_{is})(RRF)(V_i)(V_s)} \qquad \text{(Eq. 3)}$$

where: A = Area of peak in sample extract.

A_{is} = Area of internal standard peak in sample extract.

I_s = Areas of internal standard injected (ng).

V_e = Total volume of extract (ml).

V_i = Volume of extract injected (μl).

V_s = Volume of sludge extracted (liter).

RRF = Relative response factor.

14.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

14.3 If the surrogate standard recoverues fall outside the limits in Section 8.2, data for all parameters in that sample must be labeled as suspect.

15. Method Performance

Performance data for the application of this method to both POTW and industrial sludges are shown in Tables 12 to 14. Table 12 shows data for B/N compounds analyzed in POTW and industrial sludges via Option A, i.e., extract cleanup by silica gel chromatography and analysis by HRGC/MS. Table 13 shows data for B/N compounds analyzed via Option B, i.e., extract cleanup by GPC and analysis by packed column GC/MS, and acidic compounds in aliquots of the same sludges. Data for B/N compound were analyzed via Option B and acidic compounds in sludges from 40 POTWs. Most of these data represent results from one laboratory. Approximately 25% of the data in Table 14 was contributed by a second laboratory.

16. References

1. "Carcinogens - Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.
2. "OSHA Safety and Health Standards, General Industry" (29CFR1910), Occupational Safety and Health Administration, OSHA 2206 (Revised January 1979).
3. "Safety in Academic Chemistry Laboratories," American Chemical Society Committee on Chemical Safety, 3rd Edition Publication (1979).
4. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.
5. Grob, K., Jr., G. Grob, and K. Grob, "Comprehensive Standardized Quality Test for Glass Capillary Columns," J. Chrom., 156, 1-20 (1978).

17. Additional Sources

1. "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants." U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268, March 1977, Revised April 1977. Effluent Guidelines Division, Washington, DC 20460.

2. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, OH 45268. In preparation.

3. "Preservation and Maximum Holding Time for the Priority Pollutants," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268. In preparation.

4. Budde, W. L., and J. W. Eichelberger, "Performance Tests for the Evaluation of Computerized Gas Chromatography/Mass Spectrometry Equipment and Laboratories," EPA-600/4-80-025, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268, p. 16, April 1980.

5. Kleopfer, R. D., "Priority Pollutant Methodology Quality Assurance Review," U.S. Environmental Protection Agency, Region VII, Kansas City, KS. Seminar for Analytical Methods for Priority Pollutants, Norfolk, VA, January 17-18, 1980, U.S. Environmental Protection Agency, Office of Water Programs, Effluent Guidelines Division, Washington, DC 20460.

TABLE 1. EXTRACTABLE ORGANIC PRIORITY POLLUTANTS

Compound	CAS No.
Acids	
2-Nitrophenol	88-75-5
4-Nitrophenol	100-02-7
Pentachlorophenol	87-86-5
Phenol	108-95-2
2,4,5-Trichlorophenol	88-06-2
4-Chloro-3-methylphenol	59-50-7
2-Chlorophenol	95-57-8
2,4-Dichlorophenol	120-83-2
2,4-Dimethylphenol	105-67-9
4,6-Dinitro-2-methylphenol	534-32-1
Bases	
Benzidine	93-87-5
3,3'-Dichlorobenzidine	92-94-1
Polycyclic Aromatic Hydrocarbons	
Acenaphthene	83-32-9
Acenaphthylene	208-96-8
Anthracene	120-12-7
Benz[*a*]anthracene	56-55-3
Benzo[*b*]fluoranthene	205-99-2
Benzo[*k*]fluoranthene	207-08-9
Benzo[*g*,*h*,*i*]perylene	191-24-2
Benzo[*a*]pyrene	50-32-8
Chrysene	218-01-9
Dibenz[*a*,*h*]anthracene	53-70-3
Fluoranthene	206-44-0
Fluorene	86-73-7
Indeno[1,2,3-*cd*]pyrene	193-39-5
Naphthalene	91-20-3
Phenanthrene	85-01-8
Pyrene	129-00-0
Phthalates	
Bis(2-ethylhexyl)phthalate	117-81-7
Butylbenzylphthalate	85-68-7
Diethylphthalate	84-66-2
Dimethylphthalate	131-11-3
Di-*n*-butylphthalate	84-74-2
Di-*n*-octylphthalate	117-84-0

(continued)

TABLE 1 (continued)

Compound	CAS No.
Chlorinated Hydrocarbons	
2-Chloronaphthalene	91-58-7
1,2-Dichlorobenzene	95-50-1
1,3-Dichlorobenzene	541-73-1
1,4-Dichlorobenzene	106-46-7
Hexachlorobenzene	118-74-1
Hexachloro-1,3-butadiene	87-68-3
Hexachloroethane	67-72-1
Hexachlorocyclopentadiene	77-47-4
1,2,4-Trichlorobenzene	129-82-1
Chloroalkyl Ethers	
Bis(2-chloroethyl)ether	111-44-4
Bis(2-chloroethoxy)methane	111-91-1
Bis(2-chloroisopropyl)ether	39638-32-9
Miscellaneous Neutrals	
4-Bromophenyl phenyl ether	101-55-3
4-Chlorophenyl phenyl ether	7005-72-3
2,4-Dinitrotoluene	121-14-2
N-Nitrosodi-n-propylamine	621-64-7
N-Nitrosodimethylamine	62-75-9
2,6-Dinitrotoluene	606-20-2
Isophorone	78-59-1
Nitrobenzene	98-95-3
N-Nitrosodiphenylamine	86-30-6
1,2-Diphenylhydrazine	122-66-7
Pesticides	
β-Endosulfan	33213-65-9
α-BHC	319-84-6
γ-BHC	58-89-9
β-BHC	319-85-7
Aldrin	309-00-2
Heptachlor	76-44-8
Heptachlor epoxide	1024-57-3
α-Endosulfan	959-98-8
Dieldrin	60-57-1
4,4'-DDE	72-55-9
4,4'-DDD	72-54-8
4,4'-DDT	50-29-3

(continued)

TABLE 1 (concluded)

Compound	CAS No.
Endrin	72-20-8
Endosulfan sulfate	1031-07-8
δ-BHC	319-86-8
Chlordane	57-74-9
Toxaphene	8001-35-2
PCB-1242	53469-21-9
PCB-1221	11104-28-2
PCB-1254	11097-69-1
PCB-1232	11141-16-5
PCB-1248	12672-29-6
PCB-1260	11096-82-5
PCB-1016	12674-11-2

TABLE 2. SUGGESTED SURROGATE COMPOUNDS

Base/neutral fraction	Acid fraction
Aniline-d_5	2-Fluorophenol
Anthracene-d_{10}	Pentafluorophenol
Benzo[a]anthracene-d_{12}	Phenol-d_5
4,4'-Dibromobiphenyl	2-Perfluoromethyl phenol
4,4'-Dibromooctafluorobiphenyl	
Decafluorobiphenyl	
2,2'-Difluorobiphenyl	
2-Fluoraniline	
1-Fluoronaphthylene	
2-Fluoronaphthylene	
Naphthalene-d_8	
Nitrobenzene-d_5	
1,2,3,4,5-Pentafluorobiphenyl	
Pyridine-d_5	

TABLE 3. DFTPP KEY IONS AND ION ABUNDANCE CRITERIA

m/e	Ion abundance criteria
51	30-60% of m/e 198
68	< 2% of m/e 69
70	< 2% of m/e 69
127	40-60% of 198
197	< 1% of mass 198
198	Base peak, 100% relative abundance
199	5-9% of m/e 198
275	10-30% of m/e 198
365	1% of m/e 198
441	Present and < m/e 443
442	40% of m/e 198
443	17-23% of m/e 442

[a] Eichelberger, J. W., L. E. Harris, and W. L. Budde, "Reference Compound to Calibrate Ion Abundance Measurement in Gas Chromatography-Mass Spectrometry," Analytical Chemistry, 47, 995 (1975).

TABLE 4. REPRESENTATIVE EXTRACTABLE COMPOUNDS FOR RECOVERY STUDIES

Acenaphthylene
Benzidine
Benzo[a]pyrene
Bis(2-chloroethyl) ether
Bis(2-chloroisopropyl) ether
Bis(2-ethylhexyl)phthalate
Butylbenzyl phthalate
3,3'-Dichlorobenzidine
2,4-Dichlorophenol
2,4-Dimethylphenol
2,6-Dinitrotoluene
Fluoranthene
Hexachloroethane
N-Nitrosodimethylamine
1,4-Dichlorobenzene
Pentachlorophenol
Phenol
Dieldrin
α-BHC
p,p'-DDE
Heptachlor

TABLE 5. ELUTION PATTERNS AND RECOVERIES OF SELECTED BASE/NEUTRALS CHROMATOGRAPHED ON SILICA GEL[a]

Compound	Spike level (μg)	Fraction I hexane (20 ml)	Fraction II 10% dichloromethane/ hexane (50 ml)	Fraction III 50% dichloromethane/ hexane (50 ml)	Fraction IV 5% acetone/ dichloromethane (150 ml)	Total Recovery[b] (%)
1,4-Dichlorobenzene	46.8		+[c]	+		70
Hexachloroethane	31.2		+			83
Bis(2-chloroisopropyl)ether)	49.6				+	85
Bis(2-chloroethyl)ether	31.6				+	93
Acenaphthylene	28.5			+		79
2,6-Dinitrotoluene	35.1				+	96
Fluoranthene	27.0			+		90
3,3'-Dichlorobenzidine	36.4				+	50
n-Butylbenzylphthalate	45.2				+	97
Bis(2-ethylhexyl)phthalate	39.6				+	93
Benzo[a]pyrene	30.0			+		78

a 3% deactivated silica gel.

b Analyses done by packed column GC/MS.

c + indicates more than 1% recovery in that fraction.

TABLE 6. ELUTION PATTERNS AND RECOVERIES OF SELECTED BASE/NEUTRALS CHROMATOGRAPHED ON FLORISIL[a]

Compound	Spike level (µg)	Fraction I hexane (20 ml)	Fraction II 10% dichloromethane/ hexane (50 ml)	Fraction III 50% dichloromethane/ hexane (50 ml)	Fraction IV 5% acetone/ dichloromethane (150 ml)	Total Recovery[b] (%)
1,4-Dichlorobenzene	93.6		+[c]	+	+	48
Hexachloroethane	62.4		+	+	+	67
Bis(2-chloroisopropyl)ether	42.2			+	+	150
Bis(2-chloroethyl)ether	66.2			+	+	48
Acenaphthylene	57.0		+	+	+	92
2,6-Dinitrotoluene	70.2				+	130
Fluoranthene	54.0		+	+	+	120
3,3'-Dichlorobenzidine	72.8				+	65
n-Butylbenzylphthalate	90.4				+	110
Bis(2-ethylhexyl)phthalate	79.2				+	92
Benzo[a]pyrene	60.0			+	+	87

a 1% deactivated florisil.

b Analyses done by packed column GC/MS.

c + indicates more than 1% recovery in that fraction.

TABLE 7. ELUTION PATTERNS AND RECOVERIES OF SELECTED BASE/NEUTRAL/PESTICIDES CHROMATOGRAPHED ON SILICA GEL WITH AN ALTERNATE ELUTION[a,b] SCHEME

Compound	Fraction I hexane (100 ml)	Fraction II 10% dichloromethane/ hexane (50 ml)	Fraction III 50% dichloromethane/ hexane (50 ml)	Fraction IV 5% acetone/ dichloromethane (150 ml)	Total Recovery[c] (%)
Bis(2-chloroethyl)ether				+[d]	95 ± 22
1,3-Dichlorobenzene	+				64 ± 6
1,2-Dichlorobenzene	+				67 ± 9
Hexachloroethane	+				73 ± 14
N-nitrosodi-n-propylamine				+	94 ± 12
Nitrobenzene			+	+	67 ± 3
Bis(2-chloroethoxy)methane				+	66 ± 5
1,2,4-Trichlorobenzene	+				70 ± 17
Naphthalene	+	+	+		58 ± 7
Hexachlorobutadiene	+				140 ± 51
Hexachlorocyclopentadiene	+				300 ± 55
2-Chloronaphthalene	+	+			94 ± 5
Acenaphthylene		+	+		77 ± 21
2,6-Dinitrotoluene				+	93 ± 19
Acenaphthalene		+	+		88 ± 12
2,4-Dinitrotoluene				+	95 ± 14
Fluorene			+		87 ± 22
4-Chlorophenylphenylether			+		110 ± 12
Diethylphthalate				+	90 ± 41
4-Bromophenylphenylether			+		120 ± 15
Hexachlorobenzene	+				85 ± 30
Phenanthrene			+		85 ± 30
Anthracene			+		83 ± 28

(continued)

TABLE 7 (continued)

Compound	Fraction I hexane (100 ml)	Fraction II 10% dichloromethane/hexane (50 ml)	Fraction III 50% dichloromethane/hexane (50 ml)	Fraction IV 5% acetone/dichloromethane (150 ml)	Total Recovery[c] (%)
Di-n-butylphthalate				*	80 ± 51
Fluoranthene			*		51 ± 20
Pyrene			*		63 ± 12
Endosulfan sulfate				*	67 ± 17
n-Butylbenzylphthalate				*	87 ± 35
Chrysene			*		72 ± 5
3,3'-Dichlorobenzidine				*	40 ± 2
Bis(2-ethylhexyl)phthalate				*	95 ± 61
Benzo[a]pyrene			*		70 ± 34
Dibenz[a,h]anthracene			*		84 ± 17
γ-BHC		*	*	*	66 ± 27
Aldrin	*				67 ± 14
Heptachlorepoxide			*	*	88 ± 3
p,p'-DDE	*	*	*		80 ± 6
Endrin				*	77 ± 18
p,p'-DDD			*		78 ± 7
p,p'-DDT			*	*	91 ± 17
α-Endosulfan			*	*	86 ± 17

[a] Data from Lopez-Avila, Viorica, Raymond V. Northcutt, Jon Onstot, and Margie Wickham. "Analysis of the NBS Sediment by the NRI Sludge Protocol," Final Report prepared for the Environmental Protection Agency under EPA Contract No. 68-03-2711 (1981).

[b] 3% reactivated silica gel.

[c] Recoveries determined in duplicate at 8-μg and 80-μg levels for each compound. Analyses done by fused silica capillary GC/MS.

[d] * indicates more than 1% recovery in that fraction.

TABLE 8. CHROMATOGRAPHIC CONDITIONS AND CHARACTERISTIC EI IONS FOR THE BASE/NEUTRAL COMPOUND ANALYZED BY CAPILLARY COLUMN GC/MS

Compound	RRT[a,b]	Characteristic EI ions (relative intensity)
N-Nitrosodimethylamine	0.04	42(100), 74(88), 44(21)
Bis(2-chloroethyl)ether	0.21	93(100), 63(99), 95(31)
1,3-Dichlorobenzene	0.22	146(100), 148(64), 113(12)
1,4-Dichlorobenzene	0.22	146(100), 148(64), 113(11)
1,2-Dichlorobenzene	0.25	146(100), 148(64), 113 (11)
Bis(2-chloroisopropyl)ether	0.29	45(100), 77(19), 79(12)
Hexachloroethane	0.29	117(100, 199(61), 201(99)
N-Nitrosodi-n-propylamine	0.31	130(22), 42(64), 101(12)
Nitrobenzene	0.32	77(100), 123(50), 65(15)
Isophorone	0.36	82(100), 95(14), 138(18)
Bis(2-chloroethoxy)methane	0.41	93(100), 95(32), 123(21)
1,2,4-Trichlorobenzene	0.42	74(100), 109(80), 145(52)
Naphthalene	0.43	128(100), 223(63), 227(65)
Hexachlorobutadiene	0.47	225(100), 223(63), 227(65)
Hexachlorocyclopentadiene	0.60	237(100), 235(63), 272(12)
2-Chloronaphthalene	0.63	162(100), 164(32), 127(31)
Acenaphthylene	0.70	152(100), 153(16), 151(17)
Dimethylphthalate	0.72	163(100), 164(10), 194(11)
2,6-Dinitrotoluene	0.72	165(100), 63(72), 121(23)
Acenaphthene	0.73	154(100), 155(95), 152(13)
2,4-Dinitrotoluene	0.78	165(100), 63(72), 121(23)
Fluorene	0.82	166(100), 165(80), 167(14)
4-Chlorophenyl phenyl ether	0.84	204(100), 206(34), 141(29)
Diethylphthalate	0.84	149(100), 178(25), 150(10)
N-Nitrosodiphenylamine[c]	0.84	169(100), 168(71), 167(50)
1,2-Diphenylhydrazine[d]	0.86	77(100), 93(58), 105(28)
4-Bromophenyl phenyl ether	0.92	248(100), 250(99), 141(45)
Hexachlorobenzene	0.93	284(100), 142(30), 249(24)
Phenanthrene	0.98	178(100), 179(16), 176(15)
Anthracene	0.99	178(100), 179(16), 176(15)
d_{10}-Anthracene	1.00	188(100), 94(19), 80(18)
Di-n-butylphthalate	1.14	149(100), 150(27), 104(10)
Fluoranthene	1.19	202(100), 101(23), 100(14)
Pyrene	1.23	202(100), 101(26), 100(17)
Benzidene	1.24	184(100), 92(24), 185(13)
Butylbenzylphthalate	1.40	149(100), 91(50)
Chrysene	1.45	228(100), 229(19), 226(23)
3,3'-Dichlorobenzidene	1.47	252(100), 254(66), 126(16)
Bis(2-ethylhexyl)phthalate	1.52	149(100), 167(31), 279(26)
Benzo[k]fluoranthene	1.62	252(100), 253(23), 125(16)

(continued)

TABLE 8 (continued)

Compound	RRT[a,b]	Characteristic EI ions (relative intensity)
Di-*n*-octylphthalate	1.63	149(100), 167(29), 279(22)
Benzo[*a*]pyrene	1.67	252(100), 253(23), 125(21)
Dibenz[*a*,*h*]anthracene	1.84	278(100), 139(31), 279(12)
Benzo[*g*,*h*,*i*]perylene	1.87	276(100), 138(37), 277(25)

[a] Relative to d_{10}-anthracene. Retention times data were not determined for the priority pollutant pesticides and a few base/neutral compounds. However, their retention properties should be roughly equivalent to those on SP-2250 (see Table 6).

[b] SE-54 WCOT glass capillary (15 m x 0.24 mm ID), He at 10 psi, program: 50°C for 4 min, then 4°C/min to 320°C.

[c] Elutes as diphenylamine.

[d] Elutes as azobenzene.

TABLE 9. COMPOSITION OF CAPILLARY COLUMN PERFORMANCE TEST MIXTURES

Test mixture	Composition	
Varian		
P/N 82-005049-01	2-octanone	0.2 µg/µl
(nonpolar)	1-octanol	0.2
	naphthalene	0.2
	2,6-dimethylphenol	0.2
	2,4-dimethylaniline	0.2
	C_{12}-alkane	0.2
	C_{13}-alkane	0.2
Alltech		
TP-5 (polar)	C_{13}-alkane	0.1 µg/µl
	C_{14}-alkane	0.1
	C_{15}-alkane	0.1
	C_{16}-alkane	0.1
	1-octanol	0.5
	5-nonanone	0.3
	2,6-dimethylaniline	0.4
	2,6-dimethylphenol	0.4
	naphthalene	0.5
Analabs		
Test probe LPK-013F	C_{12}-acid methyl ester	41 ng/µl
(general purpose)	C_{11}-acid methyl ester	41
	C_{10}-acid methyl ester	42
	C_{10}-alkane	28
	C_{11}-alkane	29
	1-octanol	36
	Nonanal	40
	2,3-butanediol	53
	2,6-dimethylphenol	32
	2,6-dimethylaniline	32
	dicyclohexylamine	31
	2-ethylhexanoic acid	38

TABLE 10. CHROMATOGRAPHIC CONDITIONS AND CHARACTERISTIC IONS FOR THE BASE/NEUTRAL AND PESTICIDE COMPOUNDS ANALYZED BY PACKED COLUMN GC/MS

Compound	RRT[a,b]	Characteristic EI ions (relative intensity)
N-Nitrosodimethylamine	0.15	42(100), 74(88), 44(21)
1,3-Dichlorobenzene	0.31	146(100), 148(64), 113(12)
1,4-Dichlorobenzene	0.33	146(100), 148(64), 113(11)
Hexachloroethane	0.35	117(100), 199(61), 201(99)
1,2-Dichlorobenzene	0.35	146(100), 140(64), 113(11)
Bis(2-chloroisopropyl)ether	0.37	45(100), 77(19), 79(12)
N-Nitrosodi-n-propylamine	0.42	130(22), 42(64), 101(12)
Nitrobenzene	0.45	77(100), 123(50), 65(15)
Isophorone	0.47	82(100), 95(14), 138(18)
Hexachlorobutadiene	0.48	225(100), 223(63), 227(65)
1,2,4-Trichlorobenzene	0.49	74(100), 109(80), 145(52)
Bis(2-chloroethoxy)methane	0.50	93(100), 95(32), 123(21)
Naphthalene	0.51	128(100), 127(10), 129(11)
Bis(2-chloroethyl)ether	0.55	93(100), 63(99), 95(31)
Hexachlorocyclopentadiene	0.60	237(100), 235(63), 272(12)
2-Chloronaphthalene	0.68	162(100), 164(32), 127(31)
Acenaphthylene	0.75	152(100), 153(16), 151(17)
Acenaphthene	0.77	154([illegible]), 153(95), 152(53)
Dimethyl phthalate	0.78	163(100), 164(10), 194(11)
2,6-Dinitrotoluene	0.81	165(100), 63(72), 121(23)
Fluorene	0.85	166(100), 165(80), 167(14)
2,4-Dinitrotoluene	0.85	165(100), 63(72), 121(23)
4-Chlorophenyl phenyl ether	0.85	204(100), 206(34), 141(29)
Diethyl phthalate	0.87	149(100), 178(25), 150(10)
1,2-Diphenylhydrazine[b]	0.88	77(100), 93(58), 105(28)
N-Nitrosodiphenylamine[c]	0.89	169(100), 168(71), 167(50)
Hexachlorobenzene	0.92	284(100), 142(30), 249(24)
4-Bromophenyl phenyl ether	0.92	248(100), 250(99), 141(45)
Phenanthrene	0.99	178(100), 179(16), 176(15)
Anthracene	0.99	178(100), 179(16), 176(15)
Deuterated anthracene (D-10)	1.00	188(100), 94(19), 80(18)
D-n-butylphthalate	1.09	149(100), 150(27), 104(10)
Fluoranthene	1.18	202(100), 101(23), 100(14)
Pyrene	1.22	202(100), 101(26), 100(17)
Benzidine	1.27	184(100), 92(24), 185(13)
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	1.33	322(100), 320(90), 59(95)
Butylbenzylphthalate	1.34	149(100), 91(50)
Bis(2-ethylhexyl)phthalate	1.37	149(100), 167(31), 279(26)
Chrysene	1.40	228(100), 229(19), 226(23)

(continued)

TABLE 10 (continued)

Compound	RRT[a,b]	Characteristic EI ions (relative intensity)
Benz[*a*]anthracene	1.40	228(100), 229(19), 226(19)
Benzo[*b*]fluoranthene	1.43	252(100), 253(23), 125(15)
Benzo[*k*]fluoranthene	1.43	252(100), 253(23), 125(16)
3,3'-Dichlorobenzidine	1.45	252(100), 254(66), 126(16)
Di-n-octylphthalate	1.50	149(100), 167, 279
Benzo[*a*]pyrene	1.50	252(100), 253(23), 125(21)
Indeno[1,2,3-*cd*]pyrene	1.86	276(100), 138(28), 277(27)
Benzo[*g*,*h*,*i*]perylene	1.98	276(100), 138(37), 277(25)
Bis(chloromethyl) ether	d	45(100), 49(14), 51(5)
β-Endosulfan	0.47	201(100), 283(48), 278(30)
α-BHC	0.94	183(100), 109(86), 181(91)
γ-BHC	1.00	183(100), 109(86), 181(91)
β-BHC	1.03	181(100), 183(93), 109(62)
δ-BHC	1.04	183(100), 109(86), 181(90)
Aldrin	1.05	181(100), 183(93), 109(62)
Heptachlor	1.06	183(100), 109(86), 181(90)
Heptachlor epoxide	1.13	66(100), 220(11), 263(73)
α-Endosulfan	1.14	100(100), 353(79), 351(60)
Dieldrin	1.18	201(100), 283(48), 278(30)
4,4'-DDE	1.20	79(100), 263(28), 279(22)
4,4'-DDD	1.22	246(100), 248(64), 176(65)
4,4'-DDT	1.27	235(100), 237(76), 165(93)
Endrin	1.30	81(100), 82(61), 263(70)
Endosulfan sulfate	1.30	272(100), 387(75), 422(25)
Chlordane	1.05-1.26	373(19), 375(17), 377(10)[e]
Toxaphene	1.12-1.35	(231, 233, 235)[f]
PCB-1242	0.86-1.14	(224, 260, 294)[f]
PCB-1254	1.09-1.30	(294, 330, 362)[f]

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[a] 3% SP-2250 on 100/120 mesh Supelcoport in a 1.8 m x 2 mm ID glass column; He at 30 ml/min. Program: 60°C for 2 min, then 8°C/min to 260°C and hold for 15 min.

[b] Elutes as azobenzene.

[c] Elutes as diphenylamine.

[d] No data.

[e] These three ions are characteristic for the α and γ forms of chlordane. No stock should be set in these three for other isomers.

[f] These ions are listed without relative intensities since the mixtures they represent defy characterization by three masses.

TABLE 11. CHROMATOGRAPHIC CONDITIONS AND CHARACTERISTIC IONS FOR THE ACID COMPOUNDS ANALYZED BY PACKED COLUMN GC/MS

Compound	RRT[a,b]	Characteristic EI ions (relative intensity)
Chlorophenol	0.51	128(100), 64(54), 130(31)
2-Nitrophenol	0.55	139(100), 65(35), 109(8)
Phenol	0.61	94(100), 65(17), 66(19)
2,4-Dimethylphenol	0.67	122(100), 107(90), 121(55)
2,4-Dichlorophenol	0.69	162(100), 164(50), 98(61)
2,4,6-Trichlorophenol	0.79	196(100), 198(92), 200(26)
4-Chloro-m-cresol	0.86	142(100), 107(80), 144(32)
2,4-Dinitrophenol	1.04	184(100), 63(59), 154(53)
4,6-Dinitro-o-cresol	1.04	198(100), 182(35), 77(28)
Pentachlorophenol	1.13	266(100), 264(62), 268(63)
4-Nitrophenol	1.63	65(100), 139(45), 109(72)

[a] Relative to d_{10}-anthracene.

[b] 1% SP-1240-DA on 100/120 Supelcoport in a 1.2 m x 2 mm ID glass column; He at 30 ml/min. Program: 85°C for 2 min, then 10°C/min to 185°C and hold for 5 min.

TABLE 12. ACCURACY AND PRECISION FOR BASE/NEUTRAL EXTRACTABLE ORGANICS ANALYZED VIA OPTION A (SILICA GEL CLEANUP, HRGC/MS DETERMINATION) IN THREE POTW AND TWO INDUSTRIAL SLUDGES

	Three POTW sludges						Two industrial sludges					
		Spike level (µg/L)		Spike recovery				Spike level (µg/L)		Spike recovery		
Compound	Determinations	Minimum	Maximum	Mean (%)	Standard deviation	Mean RSD for Tripl. (%)	Determinations	Minimum	Maximum	Mean (%)	Standard deviation	Mean RSD for Tripl. (%)
1,4-Dichlorobenzene	27	400	40,000	44	25	37	18	400	40,000	65	59	32
Hexachloroethane	25	400	40,000	24	17	33	18	400	40,000	55	65	55
Bis(2-chloroethyl)ether	27	400	40,000	110	77	54	16	400	40,000	80	44	39
Acenaphthylene	27	400	40,000	110	66	26	15	400	40,000	95	27	14
2,6-Dinitrotoluene	25	400	40,000	33	35	72	15	400	40,000	89	63	42
Fluoranthene	24	400	40,000	140	64	25	15	400	40,000	110	42	18
Benzidine	18	7,900	80,000	31	31	49	12	700	80,000	120	96	25
3,3'-Dichlorobenzidine	9	402	4,130	50	78	66	a	-	-	-	-	-
Butylbenzylphthalate	18	4,000	40,000	140	59	18	12	400	40,000	130	92	19
Di-*n*-octylphthalate	18	400	40,000	130	75	40	15	400	40,000	130	71	30
Benzo[*a*]pyrene	21	400	40,000	110	43	26	15	400	40,100	82	70	30

a This compound was not spiked into the industrial sludges.

TABLE 13. ACCURACY AND PRECISION FOR BASE/NEUTRAL EXTRACTABLE ORGANICS ANALYZED VIA OPTION B (GPC CLEANUP, GC/MS DETERMINATION AND ACIDIC EXTRACTABLE ORGANICS IN THREE POTW AND TWO INDUSTRIAL SLUDGES

	Three POTW sludges						Two industrial sludges					
				Spike recovery						Spike recovery		
		Spike level (µg/L)						Spike level (µg/L)				
Compound	Determinations	Minimum	Maximum	Mean (%)	Standard deviation	Mean RSD for Tripl. (%)	Determinations	Minimum	Maximum	Mean (%)	Standard deviation	Mean RSD for Tripl. (%)
1,4-Dichlorobenzene	27	40	41,600	69	28	16	15	400	40,000	75	16	9
Hexachloroethane	23	400	44,100	48	17	10	16	400	40,000	56	31	14
Bis(2-chloroethyl)ether	25	400	40,700	220	190	17	16	400	40,000	120	51	7
Acenaphthylene	21	400	40,000	65	26	9	18	400	40,000	110	47	12
2,6-Dinitrotoluene	14	4,000	40,000	50	31	10	12	4,000	40,000	100	35	10
Fluoranthene	24	400	40,000	51	27	14	15	400	40,000	91	22	8
Benzidine	14	4,820	80,000	79	59	8	16	800	70,000	77	41	10
3,3'-Dichlorobenzidine	13	415	4,130	69	40	11	3	4,000	4,000	110	13	13
Butylbenzylphthalate	18	4,500	40,000	130	120	7	12	400	40,000	69	15	9
Di-n-octylphthalate	28	4,000	40,000	98	77	13	12	400	40,000	81	73	10
Benzo[a]pyrene	16	400	40,600	120	94	13	16	400	40,000	66	47	7
Phenol	24	400	46,300	73	26	22	14	400	40,000	72	34	26
2,4-Dimethylphenol	26	400	41,100	58	62	37	12	400	40,000	110	64	23
2,4-Dichlorophenol	21	399	40,000	89	32	18	15	400	40,000	140	96	15
Pentachlorophenol	26	400	40,000	120	67	37	10	400	40,000	50	16	28

TABLE 14. ACCURACY AND PRECISION FOR BASE/NEUTRAL EXTRACTABLE ORGANICS ANALYZED VIA OPTION B (GPC CLEANUP, GC/MS DETERMINATIONS) AND ACIDIC EXTRACTABLE ORGANICS IN PRIMARY SLUDGES FROM 40 POTWs[a]

		Spike recovery[b]		
Compound	Determinations	Mean (%)	Standard Deviation	Mean Avg. Rel. Dev. for Dupl.
1,3-Dichlorobenzene	27	86	39	11
1,2-Dichlorobenzene	24	68	25	11
Nitrobenzene	13	49	28	17
Hexachlorobutadiene	27	71	22	7
Naphthalene	27	94	37	8
2-Chloronaphthalene	27	89	33	8
Acenaphthalene	24	79	27	8
2,6-Dinitrotoluene	23	59	38	15
Fluorene	27	94	34	10
N-Nitrosodiphenylamine	24	140	84	11
4-Bromophenylphenylether	27	80	27	5
Di-n-butylphthalate	26	79	43	9
Fluoranthene	26	85	40	9
Pyrene	27	90	47	8
Butylbenzylphthalate	26	95	64	11
Bis(2-ethylhexyl)phthalate	22	65	34	8
Benzo[a]pyrene	27	85	49	11
1,4-Dichlorobenzene	27	82	34	11
Hexachloroethane	21	49	25	21
Isophorone	27	51	27	15
1,2,4-Trichlorobenzene	27	83	32	9
Bis(2-chloroethoxy)methane	24	54	27	12
Hexachlorocyclopentadiene	20	0	0	0
Acenaphthylene	19	76	24	9
Dimethylphthalate	27	50	33	20
2,4-Dinitrotoluene	26	65	50	14
Diethylphthalate	27	65	35	12
Hexachlorobenzene	27	63	21	10
Phenanthrene/anthracene	26	71	39	11
Chrysene/Benz[a]anthracene	27	71	27	11
Benzo[g,h,i]perylene	19	31	15	10
N-Nitrosodi-N-propylamine	24	77	54	13
1,2-Diphenylhydrazine	23	64	30	9
Benzidine	21	8	11	15
3,3'-Dichlorobenzidine	27	56	58	14
2-Chlorophenol	27	59	16	14
2-Nitrophenol	27	46	39	17
4-Nitrophenol	25	22	23	15
Phenol	26	66	28	11

(continued)

TABLE 14 (continued)

Compound	Determinations	Spike recovery[b] Mean (%)	Standard Deviation	Mean Avg. Rel. Dev. for Dupl.
2,4-Dimethylphenol	27	34	39	20
2,4-Dichlorophenol	27	68	25	11
2,4,6-Trichlorophenol	27	71	25	13
1,3-Dichlorobenzene	27	86	39	11
p-Chloro-*m*-cresol	22	58	32	9
2,4-Dinitrophenol	24	14	27	11
4,6-Dinitro-*o*-cresol	26	13	24	15
Pentachlorophenol	27	84	43	15
α-BHC	27	39	32	10
Dieldrin	27	72	30	8
4,4'-DDE	27	57	19	11
Heptachlor	26	76	35	10

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[a] Swanson, Stephen E., T. Murry Williams, Lloyd M. Petrie, and Earl M. Hansen, "Survey of Analysis of POTW Sludges for Priority Pollutants: Part II Quality Assurance Data," Final Report prepared under EPA Contract No. 68-01-5915, Task 35 (1981).

[b] Minimum spike level 125 μg/L; maximum spike level 1,250 μg/L.

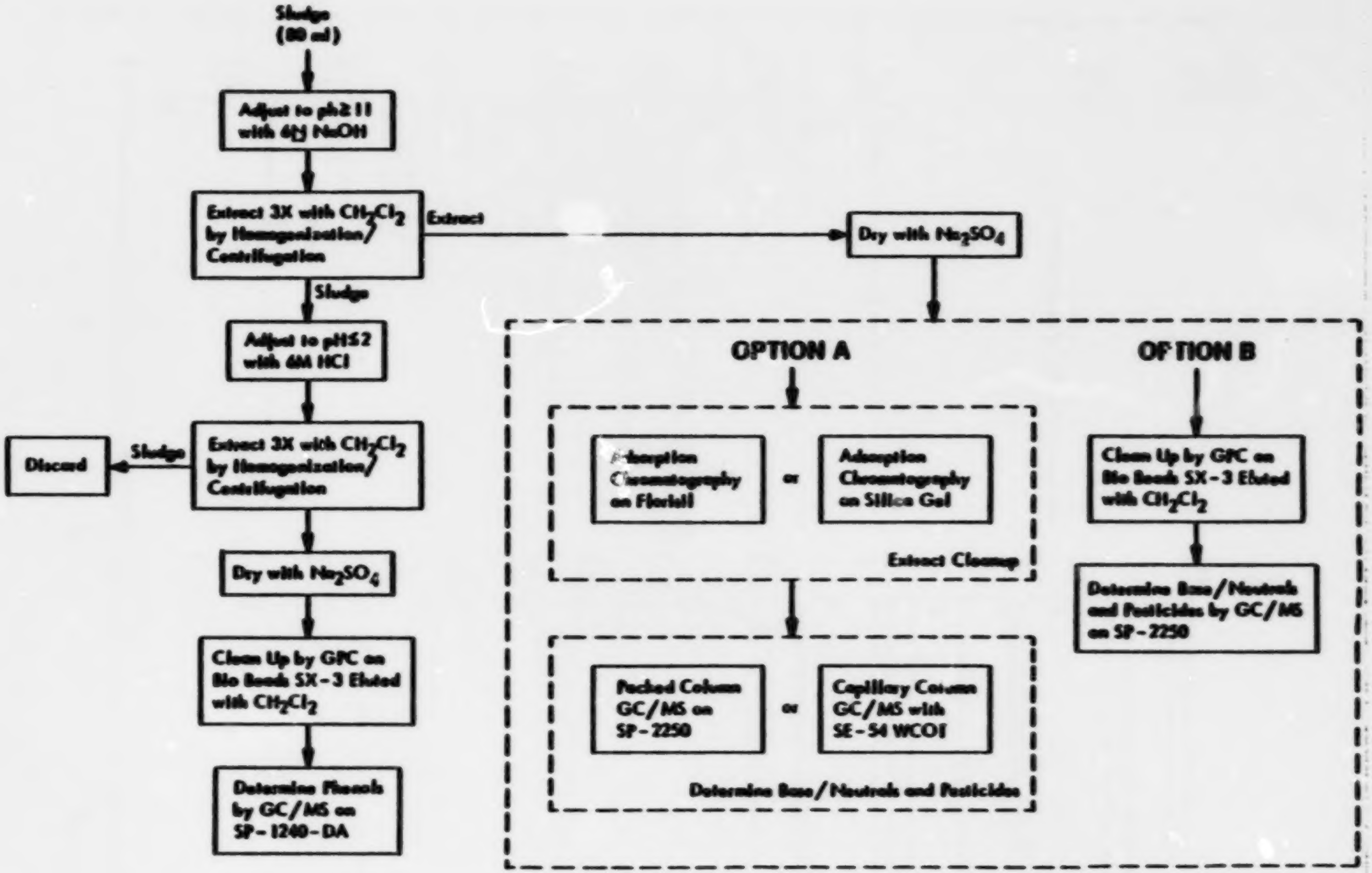
Sludge (80 ml)
Adjust to pH≥11 with 6N NaOH
Extract 3X with CH2Cl2 by Homogenization/Centrifugation
Extract
Sludge
Adjust to pH≤2 with 6M HCl
Extract 3X with CH2Cl2 by Homogenization/Centrifugation
Sludge
Discard
Dry with Na2SO4
Clean Up by GPC on Bio Beads SX-3 Eluted with CH2Cl2
Determine Phenols by GC/MS on SP-1240-DA
Dry with Na2SO4
OPTION A
Adsorption Chromatography on Florisil
or
Adsorption Chromatography on Silica Gel
Extract Cleanup
Packed Column GC/MS on SP-2250
or
Capillary Column GC/MS with SE-54 WCOT
Determine Base/Neutrals and Pesticides
OPTION B
Clean Up by GPC on Bio Beads SX-3 Eluted with CH2Cl2
Determine Base/Neutrals and Pesticides by GC/MS on SP-2250

Figure 1. Scheme for analysis of extractable organics in sludge.

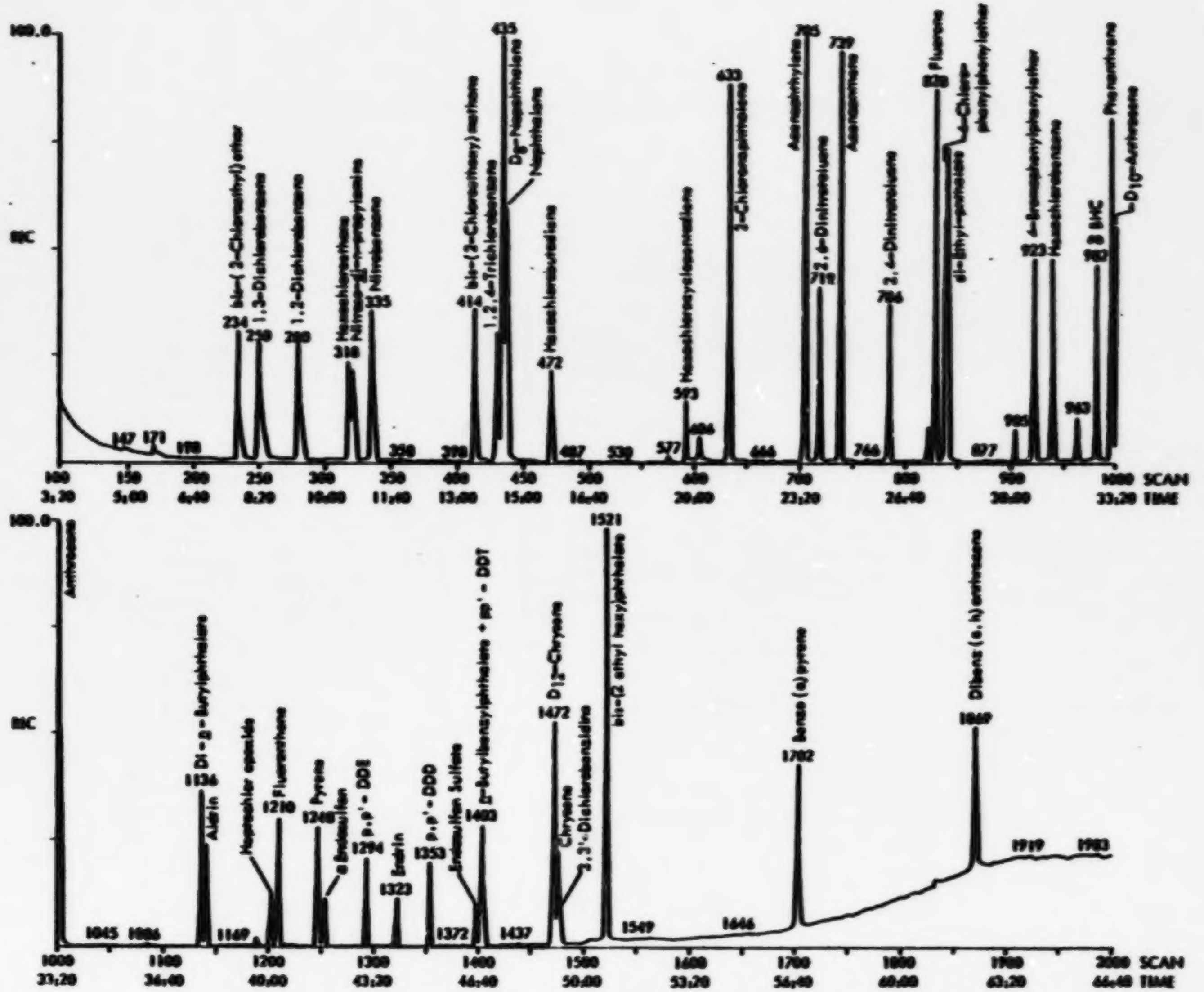

Figure 2. GC/MS chromatogram of base/neutral compounds on a fused silica capillary column.

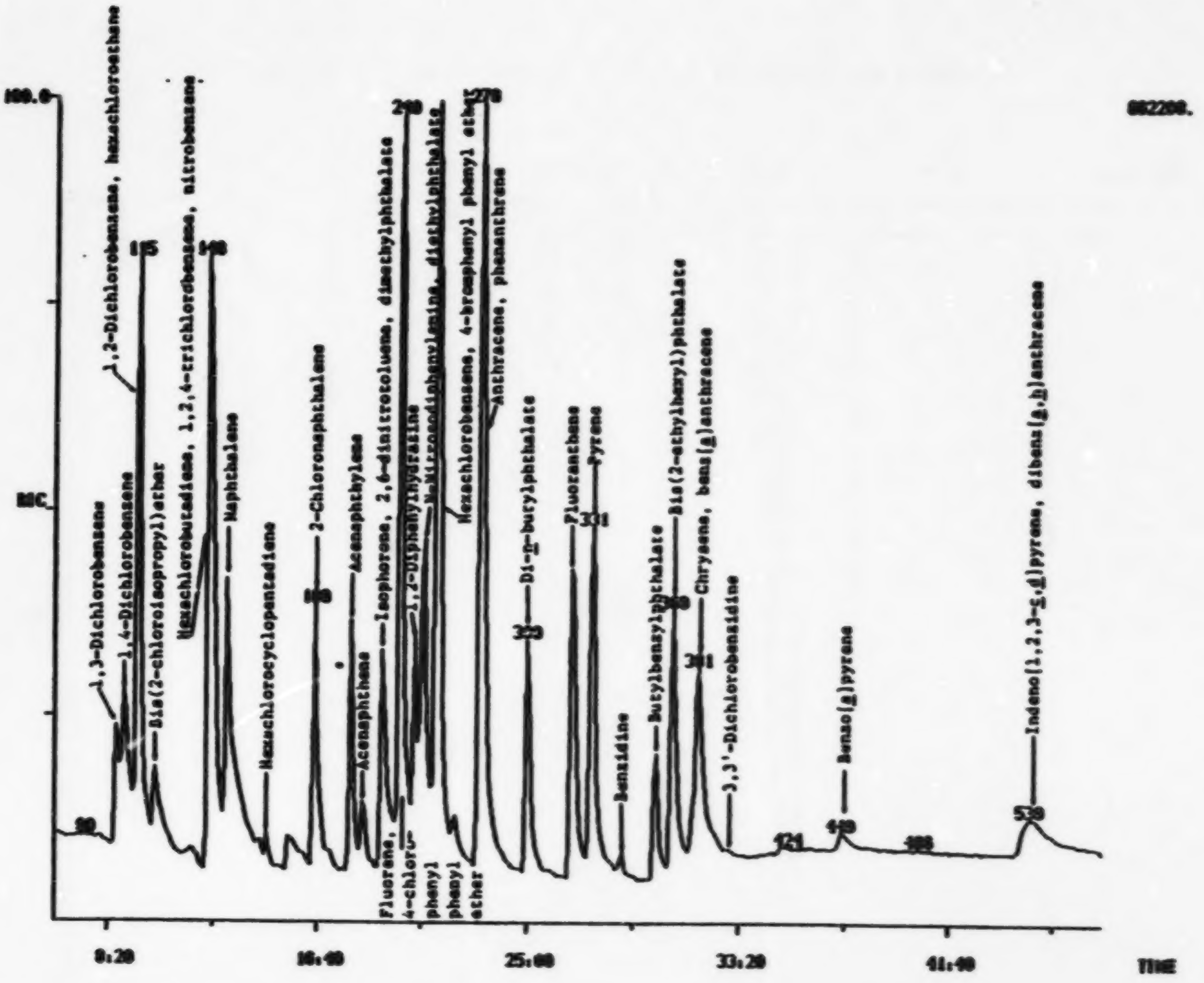

Figure 3. GC/MS chromatogram for the base/neutral compounds on a packed column.

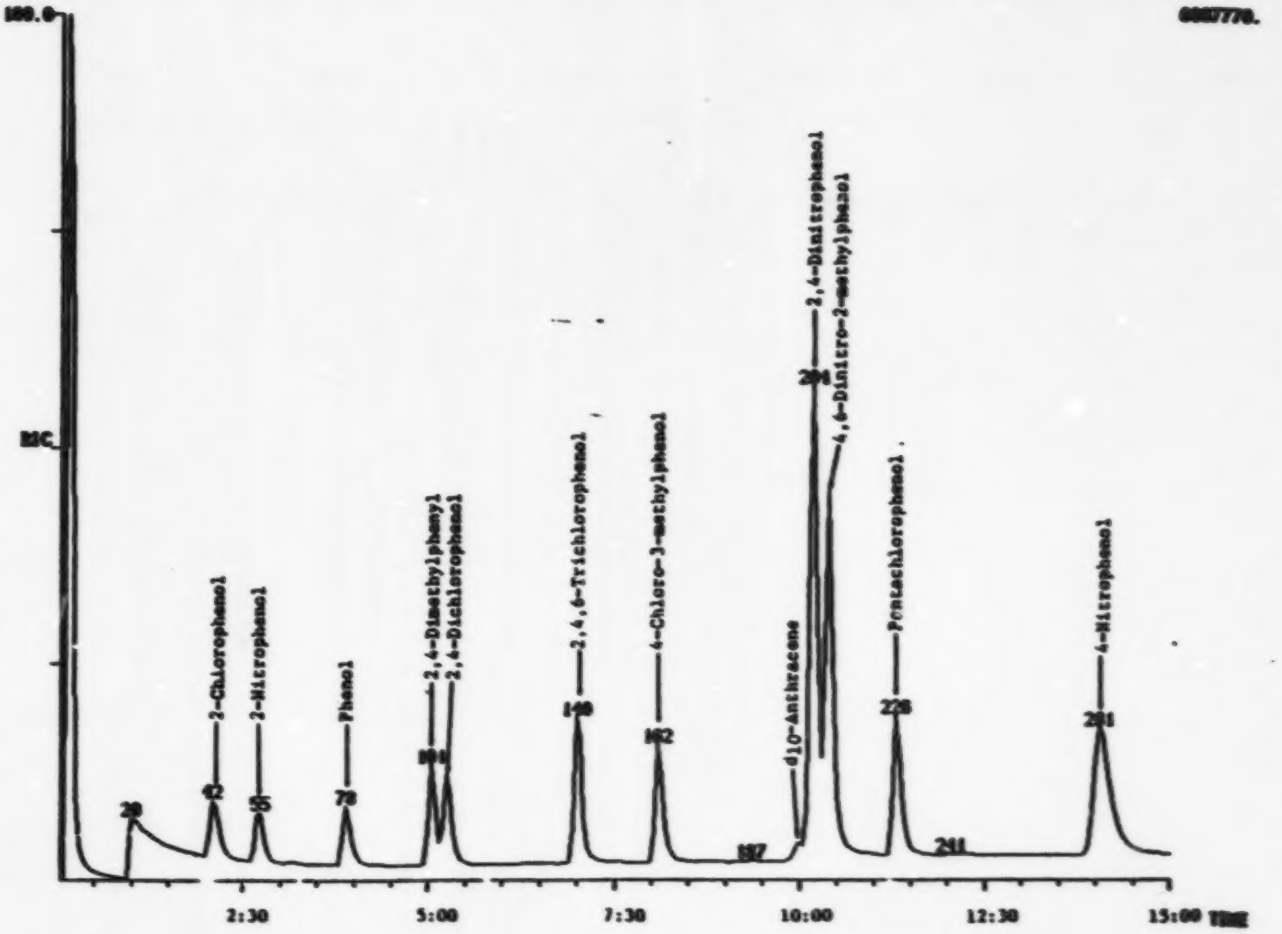

Figure 4. GC/MS chromatogram of the acidic compounds.